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Atlantic Salmon Brood Stock Management and Breeding Handbook

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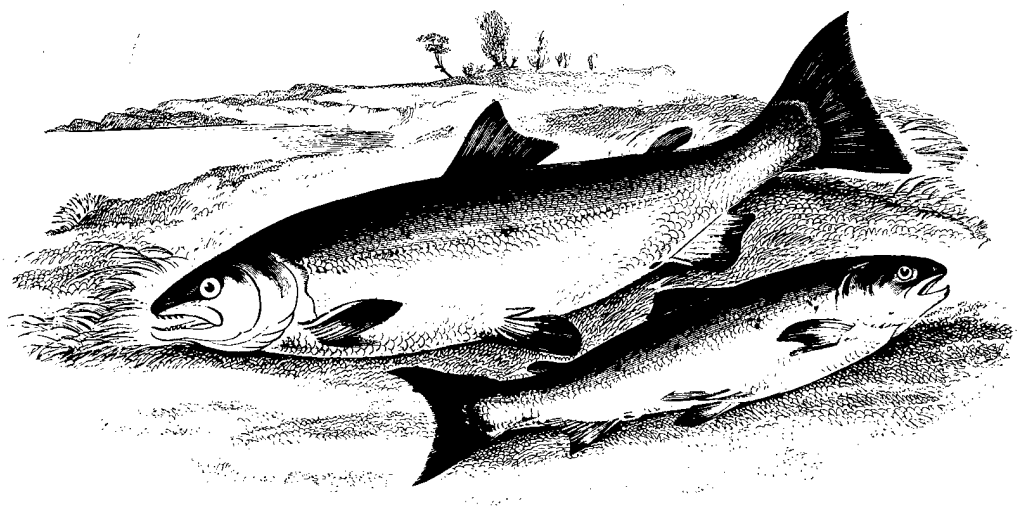

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Atlantic Salmon Brood Stock Management and Breeding Handbook

edited by

Harold L. Kincaid

U.S. Fish and Wildlife Service
National Fishery Research and Development Laboratory
R.D.# 4, Box 63
Wellsboro, PA 16901

and

Jon G. Stanley

U.S. Fish and Wildlife Service
National Fisheries Research Center– Great Lakes
1451 Green Road
Ann Arbor, MI 48105

U.S. Department of the Interior
Fish and Wildlife Service
Washington, DC 20240

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Preface

The Atlantic salmon stock selection workshop was held 27–28 September 1983 at Nashua, New Hampshire, for Federal and State agencies participating in programs to restore Atlantic salmon in the northeastern United States. Genetic implications of hatchery procedures and management practices used throughout the restoration program were discussed at length. Evolution of the restoration program was reviewed to examine progress in strain selection methods and to evaluate the relative success of each method.

The group attending this workshop decided that an Atlantic salmon brood stock management and breeding handbook was needed to provide standardized guidelines on the handling and management of Atlantic salmon brood stocks. This handbook summarizes the workshop and reviews current practices used by agencies participating in the Atlantic salmon restoration program.

Initially, the restoration program relied on eggs imported from Canada to supplement those available in New England from small residual anadromous and landlocked populations. Eggs were placed in hatchery facilities for incubation, and fingerlings were reared for reintroduction into the rivers as smolts. Over the years many sources of eggs were used in the restoration program – Canadian sea-run strains, landlocked strains, hatchery brood stocks, and strain hybrids. More recently, the Penobscot River has been the primary source of eggs for stocking all New England rivers.

Since the mid-1960's, Atlantic salmon culture has become more efficient. Many new cultural practices have contributed to this improved efficiency, including modification of water temperatures to accelerate egg incubation and early fry growth, production of 1-year smolts using accelerated growth techniques, development of an Atlantic salmon diet, establishing domestic brood stocks, techniques for holding kelt brood stocks, and improvement of fry stocking techniques. However, the potential of these practices to change the genetic constitution of natural populations has not been evaluated. To address the problem of the effect of hatchery practices on natural gene pools, certain questions must be asked: Does the practice result in the selective loss of a fraction of the gene pool? Does the practice discriminate against specific performance or behavioral traits? How would the total or partial loss of fish expressing a specific trait affect the long-term restoration of the fishery to historic levels? What is the genetic variability of the population? What is the effective population size? What is the potential for inbreeding? Can (should) the genetic variability of current brood stocks be increased? These questions and others were discussed throughout the workshop and summarized in this handbook.

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Chapter 1. History of the Atlantic Salmon Restoration Program

by

Stephen Rideout

U.S. Fish and Wildlife Service
Division of Fish and Wildlife Management Assistance
Arlington Square Building, Mail Stop 840
18th and C Streets, NW.
Washington, DC 20240

Federal and State agencies are attempting to restore Atlantic salmon (*Salmo salar*) runs in all New England States. Historically, most rivers in New England supported Atlantic salmon, with the largest runs in the Androscoggin, Connecticut, Kennebec, Merrimack, and Penobscot systems. By the mid-1980's, cooperative restoration programs had reestablished runs in the Connecticut, Merrimack, Pawcatuck, Penobscot, and St. Croix rivers (Table 1.1). More than 90% of the adult fish returning to the Penobscot River were stocked as hatchery smolts, whereas all adult fish returning to the Connecticut, Merrimack, and Pawcatuck rivers were from plantings of hatchery smolts, parr, or fry.

Early restoration efforts in Maine relied on salmon fry, parr, and smolts cultured from eggs taken from the Miramichi River (Saunders 1981). These introductions were not successful, and adult returns did not improve until eggs from residual populations from the Machias and Narraguagus rivers were used. Currently, brood fish for the Penobscot River are trapped in the lower main stem of the river. Additional brood fish are trapped in the nearby Union River as adult returns from cultured Union River smolts.

More than 8.9 million smolts, parr, and fry in various combinations have been stocked into the Penobscot River drainage since 1948 (Table 1.2). Penobscot River stocking has emphasized hatchery smolt production using both 1- and 2-year-old smolts. One-year smolts have dominated the stockings since 1983. Eggs for hatchery production have been obtained from sea-run adults returning to the Penobscot River.

The Merrimack River has been stocked annually with fry, parr, and smolts since 1975. As with most rivers in southern New England, eggs were obtained from many

Table 1.1. *Annual return of adult Atlantic salmon to three New England river systems, 1975–87.*

Year	River system		
	Connecticut	Merrimack	Penobscot
1975	—	—	1,006
1976	—	—	673
1977	—	—	644
1978	93	—	1,824
1979	58	—	918
1980	175	—	3,327
1981	529	—	3,455
1982	70	23	4,161
1983	39	114	974
1984	92	104	1,845
1985	310	212	3,362
1986	318	103	4,529
1987	353	139	2,474

Table 1.2. *Numbers of Atlantic salmon stocked into the Penobscot River, Maine, 1948–87.*

Year	Group size			
	Fry	0 Parr	1 Parr	Smolts
1948	—	61,000	—	—
1949	—	38,057	15,191	—
1950	—	29,547	39,210	—
1954	—	33,350	68,317	—
1955	—	68,492	—	—
1956	—	79,316	—	—
1957	—	—	97,896	—
1958	—	—	42,500	—
1959	—	—	50,596	—
1962	—	—	—	34,000
1965	—	—	—	55,890
1966	—	—	—	7,005
1967	—	—	—	43,177
1968	—	—	—	48,698
1969[a]	—	—	25,000	27,821
1970	—	25,000	—	28,497
1971	—	—	15,800	68,387
1972	129,000	—	—	73,791
1973	—	—	—	109,080
1974	—	—	44,184	100,241
1975	—	8,200	4,105	110,556
1976	—	—	185,958	226,918
1977	—	—	—	340,803
1978	—	—	116,398	210,190
1979	28,144	66,581	—	292,708
1980	—	—	—	584,201
1981	201,779	25,370	50,257	199,555
1982	248,150	50,933	206,430	315,704
1983	—	—	31,925	445,898
1984	80,050	34,362	—	617,666
1985	196,800	59,450	17,575	580,464
1986	225,750	25,700	58,850	589,204
1987	304,976	58,131	101,081	539,175
Total	1,450,649	663,489	1,171,273	5,649,629

[a] First year that brood stock captured on the Penobscot River supplied all eggs for the smolt production program.

sources including pure Canadian strains, the Penobscot River, and landlocked salmon eggs fertilized with milt from sea-run Penobscot fish. Since 1983, eggs have been taken from adults returning to the Merrimack River to partly supply the culture needs of the Nashua National Fish Hatchery.

The Pawcatuck River in Rhode Island has been stocked with 0+ or 1+ parr (the + indicates that the fish had hatched before the beginning of the year when the year class is said to form) from strains from the Penobscot and Union rivers (Table 1.3). Crosses of landlocked × sea-run (Penobscot) salmon were stocked in 1979, and 1,000 0+ pure-strain Canadian pre-smolts were stocked in 1982. Eggs from returning Pawcatuck adults were first taken in 1982.

Fry, parr, and smolts have been planted annually in the Connecticut River; however, the primary emphasis has been on hatchery smolts. During 1977–80, smolts were produced from a variety of egg sources (Table 1.4). More recently, Connecticut River and Penobscot River sea-run or Green Lake domestic (Union River) brood stock supplied most eggs needed by the Atlantic salmon restoration program. The current plan requires that adults returning from the Connecticut River be the primary source of eggs, to the extent that they are available.

Table 1.3. *Summary of Atlantic salmon releases, smolt production, and adult returns in the Pawcatuck River, 1979–88.*

Year	Fish stocking					Populations	
	0 Fry	0 Parr	1 Parr	Hatchery smolt	Total	Wild smolts[a]	Adult return
1979	0	136,000	0	0	136,000	0	0
1980	0	1,000	0	0	1,000	1,582	0
1981	0	2,000	108,000	800	110,000	3,788	0
1982	2,200	1,000	0	0	3,200	7,795	38
1983	0	650	0	0	650	115	38
1984	0	23,000	0	0	23,000	83	26
1985	8,000	51,032	1,400	0	60,432	5,032	1
1986	0	50,741	15,000	0	65,741	5,401	0
1987	3,000	46,240	4,660	1,000	54,900	7,898	1
1988	150,000	75,000[b]	7,282[b]	5,400[b]	227,500	7,020	6
Total	163,200	386,663	136,342	7,200	682,423	38,714	110

[a] Estimated production of wild smolts from stream surveys at 15 index stations.
[b] Projected from North Attleboro National Fish Hatchery and Perryville State Fish Hatchery.

To augment egg supplies of strains from the Connecticut and Merrimack rivers, adult females have been reconditioned after spawning and domestic brood stock has been developed. The first significant use of eggs from adults returning to the Connecticut River was in 1979. These eggs produced 32,600 2-year-old smolts for stocking in 1982 (Table 1.4). Currently, most hatchery smolt production in the Connecticut River is aimed at producing 1-year smolts exceeding 15 cm length at stocking. The goal of the Connecticut River program is to annually produce 4.6 million eggs, of which 1.3 million would be for smolt production and 3.3 million would be for fry stocking.

Data are needed to evaluate the relative efficiency of fry, parr, and smolt stockings for production of adult returns. Preliminary information from the Merrimack and Pawcatuck rivers suggests that marine survival of smolts from fry or parr plants is higher than that for hatchery smolts. Survival from egg to smolt, however, is higher for hatchery smolts; therefore, adult returns per 1,000 eggs generally are higher for hatchery smolts.

Table 1.4. *Strains of Atlantic salmon stocked in the Connecticut River system, number stocked in thousands.*

Year/stage	Strain[a]								Strain hybrids			
	A	B	C	D	E	F	G	H	A×B	B×J	B×H	A×J
1977												
Fry	—	—	—	—	—	50.0	—	—	—	—	—	—
Parr	—	—	—	—	—	—	—	—	—	—	—	—
Smolt	—	5.4	—	—	4.5	7.5	—	—	—	5.2	91.7	—
1978												
Fry	—	—	—	—	27.4	22.5	—	—	—	—	—	—
Parr	—	—	—	—	—	—	—	—	—	—	—	—
Smolt	—	7.1	6.2	—	58.3	—	—	42.2	—	17.0	—	—
1979												
Fry	—	—	—	—	—	24.5	190.0	29.0	—	91.0	—	—
Parr	—	—	—	—	—	—	—	—	—	—	—	—
Smolt	—	132.2	—	—	—	—	—	18.5	19.0	—	—	—
1980												
Fry	—	153.0	—	—	—	—	19.7	—	—	—	—	—
Parr	—	—	—	—	—	—	—	—	—	—	—	—
Smolt	0.9	3.7	—	—	—	—	—	11.5	3.1	44.3	—	—

Table 1.4. *Continued.*

1981												
Fry	39.6	264.2	—	—	—	—	—	—	—	—	—	—
Parr	46.0	136.0	—	—	—	—	—	—	—	—	—	—
Smolt	—	29.8	—	—	—	—	—	54.1	0.7	—	—	—
1982												
Fry	—	—	—	—	—	—	—	—	—	—	—	—
Parr	—	19.4	—	—	—	—	—	—	—	—	—	—
Smolt	32.6	195.0	—	—	—	—	—	—	—	—	—	7.2
1983												
Fry	8.0	—	45.0	—	—	—	160.9	16.7	—	—	—	—
Parr	184.3	—	36.6	—	—	—	—	67.8	—	—	—	—
Smolt	189.5	—	7.1	—	—	—	—	—	—	—	—	9.5
1984												
Fry	64.5	32.4	—	528.0	—	—	—	—	—	—	—	—
Parr	67.6	153.7	—	94.6	—	—	—	76.2	—	—	—	—
Smolt	46.4	147.3	—	97.3	—	—	—	20.8	—	—	—	—
1985												
Fry	—	64.0	—	358.1	—	—	—	—	—	—	—	
Parr	83.1	35.6	—	106.7	—	—	—	0.7	—	—	—	—
Smolt	65.4	130.2	—	76.9	—	—	—	10.8	—	—	—	—
1986												
Fry	—	—	—	154.1	—	—	—	—	7.7	—	—	—
Parr	361.5	21.7	—	87.5	—	—	—	—	—	—	—	—
Smolt	206.3	31.6	—	64.3	—	—	—	—	—	—	—	—
1987												
Fry	246.6	99.3	—	755.2	—	—	—	—	—	—	—	—
Parr	585.1	2.9	—	140.7	—	—	—	—	—	—	—	—
Smolt	124.9	2.4	—	78.6	—	—	—	—	—	—	—	—
Totals												
Fry	358.7	612.9	45.0	1,795.4	27.4	97.0	370.6	45.7	7.7	91.0	—	—
Parr	1,327.6	369.3	36.6	429.5	—	—	—	144.7	—	—	—	—
Smolt	666.0	684.7	13.3	317.1	62.8	7.5	—	157.9	22.8	66.5	91.7	16.7

[a] Strain identification: A = Connecticut River; B = Penobscot River; C = Magaguadavic River; D = Union River; E = Miramichi River; F = Quebec; G = Icelandic; H = Other stocks: Big Salmon (1978, 1979), St. John (1980), unknown Canadian (1979, 1981, 1983, 1984, 1985); and J = landlocked salmon.

Chapter 2. Fish Breeding Principles

by

John Bailey

Atlantic Salmon Federation
P.O. Box 429
St. Andrews, New Brunswick
Canada E0G 2X0

and

Harold L. Kincaid

U.S. Fish and Wildlife Service
National Fishery Research and Development Laboratory
R.D.# 4, Box 63
Wellsboro, PA 16901

Fish breeding is the sum of all procedures necessary to culture, manage, and spawn brood stocks that lead to the production of successive generations of progeny. Fish breeding as a science began with the culture of carp in eastern Asia and has been practiced for about 4,000 years (Bardach et al. 1972). Knowledge of the management of fish brood stocks advanced slowly until about 1960 when scientific breeding principles developed in domestic animals were applied to fish species. Since 1960, research has been directed toward hatchery populations with only limited attention to natural populations. Although the management objectives for domestic and natural populations may be quite different, genetic principles are equally applicable to both. To understand the genetic principles of fish breeding, a person needs a knowledge of selection and genetic diversity. These two terms are often confused; therefore, we present a detailed discussion on these concepts. Other terms frequently used in breeding work are defined in the glossary of this handbook.

Selection and Genetic Diversity

Selection occurs when there are differential rates of reproduction among fish in a population. The driving force is known as selection pressure, where fish with certain genotypes are able to survive and reproduce at a higher rate than other individuals. Selection occurs continually in all populations. The specific traits displayed by the favored individuals depend on a variety of forces that are determined by both the environment and the fish culturist. Thus, selection can be divided into two types, natural selection and artificial selection.

Natural selection theory is closely allied with Darwin's theory of survival of the fittest – natural selection involves environmental factors acting on the individual fish in a population. Natural selection modifies populations for survival in a particular environment by eliminating individuals from the breeding population that are unable to compete successfully for food or space, avoid predators, or reproduce effectively. To the extent that these traits are determined by genes and gene combinations, natural selection tends to increase the frequency of alleles that contribute to improved fitness and decrease the frequency of alternate alleles. Because any environment varies both within and between years for such characteristics as temperature, water quality, food availability, and periodic flooding, natural selection pressure also varies in intensity and direction. Because of this, natural selection pressure tends to maintain relatively high levels of genetic variability within a population. The ultimate effect is selection for those fish that survive within the range of the fluctuating environment.

Artificial selection generally refers to the pressure applied to a breeding population to improve the expression of specific traits that are considered important by the brood stock manager. Ideally, both the direction and intensity of artificial selection pressures are determined by the culturist. A critical step in fish breeding

is the choice of adults that will be used for reproduction to contribute progeny to the next generation. Individuals or families are chosen that express improved performance for the desired traits, and only those fish are used in the production of future brood stock. In response to the mating of superior brood stock, the frequency of alleles that contribute to improved performance is expected to increase in the progeny generation. Thus, genetic improvements are made from one generation to the next. If the genetic variables and selection pressures are known, the magnitude of the response to selection can be predicted. A detailed review of selection theory can be found in Falconer (1981).

Artificial selection may also be inadvertent. In such cases neither direction nor the intensity of selection is known in advance. Inadvertent selection occurs when traits are correlated or associated with one another. When a genetic relation exists between two traits, improvement in one trait may lead to an unexpected correlated response in the other. The correlation can be positive if both traits increase, or negative if an increase in one trait causes a decrease in the other. The latter case causes difficulty during selection. For example, large egg size and high fecundity might be considered advantageous traits for salmon brood stock. However, in fish of constant size, eggs must be fewer in number to be relatively larger. Therefore, a naive attempt to improve either trait may lead to a reduction in the other. If the nature of the correlation is known, then the correlated response to selection can be predicted for either trait. Furthermore, it is possible to improve two negatively correlated traits using index selection techniques.

Genetic changes can also result from a rapidly changing environment, such as would be caused by habitat alteration. Examples include human modification of the natural habitat or moving the fish to an alternate environment such as a hatchery. From a genetic point of view the distinction between artificial and natural selection is unclear. By convention, all selection pressures derived from the environment are considered to be natural selection, even though that environment may have been artificially created or changed by humans.

All populations undergoing artificial selection are simultaneously subjected to natural selection pressures. How these two types of selection will interact cannot be predicted exactly. However, as a general rule, natural selection will partly nullify or counterbalance artificial selection that attempts to proceed at the detriment of natural fitness. The protected environment of the hatchery ensures high fry survival and favors those individuals that readily adapt to commercial diets. Those fish that do not or cannot adapt to the hatchery usually die before or during the fry stage. In the wild, fish that avoid predation and vigorously compete for available food and space are favored. Because these two survival strategies differ, natural and artificial selection are antagonistic for fish reared in hatcheries and then stocked into the wild. As fish are stocked at advancing life stages – egg, alevin, parr, smolt, or adult – the conflicting pressures become progressively greater. The potential effect of antagonism between natural and artificial selection should be considered in the development of any fishery management program that depends on hatchery production.

Domestication is the combined result of natural selection (that predisposes the population for survival in a modified and controlled environment) and artificial selection (for various traits). Domestication is characterized by genetic changes in behavior, morphology, and physiology. Therefore, domestication selection is natural selection for increased fitness in domestic environments. Doyle (1983) provided theoretical evidence that domestication selection pressure in closed fish populations may be of the same magnitude as artificial selection.

Brood stock population size is a primary consideration in the development of a breeding and selection program. In practical terms, brood stock population size is dictated by the number of eggs (progeny) required to produce future brood stocks and to meet production requirements. However, requirements based on egg needs alone can lead to genetic problems, especially in small hatchery operations; because of the high fecundity of Atlantic salmon, few females are needed as replacement brood stock. Hence, genetic diversity may be low. An important question for the brood stock manager should be: How large does the population need to be to provide a stable genetic equilibrium of gene and genotype frequencies and to avoid or minimize the risk of inbreeding over a series of generations?

Inbreeding

Inbreeding results from the mating of related individuals. The level of inbreeding depends on the closeness or degree of relatedness between mating pairs, which, in turn, depends on the number of individuals that successfully reproduced and contributed to the progeny generation. Every fish has two parents, four grandparents, eight great-grandparents, and T generations back it has 2^T ancestors. Therefore, few ancestor generations are required before the number of individuals necessary to provide separate ancestors for each fish in the current generation becomes larger than any real population could contain. Therefore, any two fish in a population must be related through one or more common ancestors. In smaller populations the common ancestors are less remote, and pairs mating at random are, on average, more closely related than in large populations.

When two individuals are related, they carry identical replicates of several alleles from their common ancestor. If they mate, these identical alleles can recombine in the offspring. Inbred fish are more likely to be homozygous than fish from unrelated parents because of the likelihood of recombination of these identical alleles. Allelic genes can be identical in two ways; therefore, homozygotes can be produced in two ways: When two alleles carried by an individual are identical in phenotypic effect and derived from different ancestors, they are called alike in state. When they are derived from a common ancestor, they are called identical by descent.

The frequency of loci that are identical by descent provides a measure of the level of inbreeding. Known as the coefficient of inbreeding and symbolized by F, it was first defined by Wright (1922) as the probability that the two alleles at any given locus in an individual are identical by descent. In other words, F reflects the probability that two gametes taken at random from different adults carry alleles at a given locus that are identical by descent. Applied to an individual, F expresses the degree of relatedness between its parents. In closed or finite populations, such as those found in hatchery brood stocks, F accumulates from one generation to the next.

Inbreeding coefficients express the level of inbreeding accumulated from a specific point in the ancestry of the population. Because the number of independent ancestors is limited in any finite population, most alleles of a single form would be identical by descent if they could be traced far enough into the past. Therefore, F is meaningful only if there is a specific generation in the past beyond which ancestries are not considered and all alleles are assumed to be independent. This arbitrary point is called the base population, and by convention the value of F in this generation is set at zero. The inbreeding coefficient of any subsequent generation expresses the degree of inbreeding that has accumulated since the base population. Reference to the base population is not always explicitly stated but is always implied.

Inbreeding depression is the reduction in mean performance in an inbred population and is measured as the differential performance between inbred and outbred groups. Inbreeding depression often is associated with traits controlling reproductive capacity and physiological efficiency. The way in which inbreeding depression occurs becomes apparent when we consider what happens in a series of 100 inbred lines created from a single base population. Collectively, these lines constitute the gene pool of the entire base population. As inbreeding advances in successive generations, random drift produces wide variation in allele frequencies among lines, including the loss of alleles from some loci. As allele frequencies among lines differentiate due to random drift and inbreeding, the phenotypic expression of traits becomes more variable. Although allele frequencies can vary widely between lines in a few generations, if there is no selection they do not change in the total population (i.e., all inbred lines collectively) as inbreeding accumulates.

The level of inbreeding depression in the total population is measured as the average reduction in performance of all the inbred lines below the mean level in the base population. Changes in the population mean, therefore, are caused by changes in genotype frequencies, and not by changes in allele frequencies, because of the increase in the frequency of homozygous genotypes. Heterozygous genotypes show a corresponding decrease in each inbred line. The effect of inbreeding, therefore, is associated with differences in the genotypic value of homozygotes as compared with heterozygotes. This example shows that a primary cause of inbreeding depression is genetic dominance and that the direction of change in phenotypic expression will be toward the value of the recessive allele.

Inbreeding depression tends to increase in proportion to the inbreeding coefficient during the initial generations. In the early generations, inbreeding may produce a deterioration in reproductive capacity, reduced viability, and ultimately the loss of some lines. When lines are lost, the surviving lines become a selected population to which the theoretical expectations no longer apply. Thus, precise measurement of the rate of inbreeding depression is limited to the first few generations, before F reaches a high level. Inbreeding not only affects the genotype of an individual, but also the maternal influence imparted to progeny during egg development. Therefore, traits affected by the maternal environment, such as hatchability, fry mortality, or fry growth, are doubly sensitive to inbreeding depression. As a result, it is difficult to show a simple linear relation between F and decreased performance in the trait measured.

Measurement of Inbreeding in Randomly Mating Populations

The average inbreeding accumulation per generation expected in randomly mating populations can be estimated by the following formula:

$$\Delta F = [1 \div 8N_m + 1 \div 8N_f] \times 100\% \qquad 2.1$$

where:

ΔF = rate of inbreeding increase per generation, expressed as percent (%),
N_m = number of males used as parents in a given generation, and
N_f = number of females used as parents in a given generation.

For example:

1. If a population is maintained with 50 males and 50 females, the inbreeding rate per generation would be

$$\Delta F = [1 \div 8(50) + 1 \div 8(50)] \times 100\%$$
$$= [1 \div 400 + 1 \div 400] \times 100\%$$
$$= 0.5\%$$

2. If a population is maintained with 10 males and 20 females, the inbreeding rate per generation would be

$$\Delta F = [1 \div 8(10) + 1 \div 8(20)] \times 100\%$$
$$= [1 \div 80 + 1 \div 160] \times 100\%$$
$$= 1.875\%$$

Inbreeding rate estimates for a variety of male and female numbers are given in Table 2.1.

Measurement Implications of Inbreeding

Formula 2.1 shows that the rate of increase in the level of inbreeding (ΔF) per generation depends on the number of individuals used as parents to produce the progeny generation and, primarily, on the sex of the parent used in the smallest numbers. For example, when only five males are used, the rate of inbreeding cannot be reduced below 2.5% per generation, irrespective of the number of females spawned (Table 2.1). In Table 2.1 the pattern prevails in both females (rows) and males (columns). Consequently, equal numbers of each sex should be used to advance the generation of all brood stock populations.

The minimum number of parents that should be used for long-term maintenance of a brood stock should be at least 50 mating pairs (i.e., 50 males and 50 females). This population size will limit the expected inbreeding accumulation rate to 0.5% per generation (Table 2.1). At 0.5% per generation, 30 generations (90–120 years in Atlantic salmon) would be required to raise inbreeding to the 15% level where inbreeding depression has been reported in rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*; Kincaid 1976). When inbreeding accumulates slowly over many generations, natural selection and mutation act to purge the most harmful gene combinations from the population. When inbreeding is rapid, however, natural selection and mutation do not have the opportunity to operate.

A common practice in many hatcheries is pooling the eggs from two to five fish and then sequentially adding milt from a similar number of males. Eggs are pooled from multiple females because it is more labor efficient. The practice of using males sequentially ensures that eggs are not wasted by mating with an infertile male. Although this good practice produces fertilized eggs efficiently, it forfeits accurate information on effective population size because the males will not be represented equally in the progeny generation. To avoid such problems, single-pair mating is recommended with family lots held separately until hatching. This allows infertile matings to be identified so that the actual number of parents contributing progeny to each successive generation will be known. It also avoids the labor of removing the large number of dead eggs—produced by infertile matings—that are mixed with eggs from fertile matings.

Table 2.1. *Increase in inbreeding coefficient (expressed in percent) per generation in randomly mating populations when different numbers of male and female parents are used to maintain the brood stock.*

	Number of male parents												
Number of female parents	1	3	5	10	20	25	50	75	100	150	200	250	∞
1	25.00	16.67	15.00	13.75	13.12	13.00	12.75	12.67	12.62	12.58	12.56	12.55	12.50
3	16.67	8.33	6.67	5.41	4.78	4.67	4.42	4.33	4.25	4.24	4.23	4.22	4.17
5	15.00	6.67	5.00	3.75	3.12	3.00	2.75	2.66	2.62	2.58	2.56	2.55	2.50
10	13.75	5.41	3.75	2.50	1.87	1.75	1.50	1.41	1.37	1.33	1.31	1.30	1.25
20	13.12	4.78	3.12	1.87	1.25	1.13	0.88	0.79	0.75	0.71	0.69	0.68	0.62
25	13.00	4.67	3.00	1.75	1.13	1.00	0.75	0.67	0.63	0.58	0.56	0.55	0.50
50	12.75	4.42	2.75	1.50	0.88	0.75	0.50	0.41	0.38	0.33	0.31	0.30	0.25
75	12.67	4.33	2.66	1.41	0.79	0.67	0.41	0.33	0.29	0.25	0.23	0.22	0.17
100	12.62	4.25	2.62	1.37	0.75	0.63	0.38	0.29	0.25	0.21	0.19	0.18	0.13
150	12.58	4.24	2.58	1.33	0.71	0.58	0.33	0.25	0.21	0.17	0.15	0.13	0.08
200	12.56	4.23	2.56	1.31	0.69	0.56	0.31	0.23	0.19	0.15	0.13	0.11	0.06
250	12.55	4.22	2.55	1.30	0.68	0.55	0.30	0.22	0.18	0.13	0.11	0.10	0.05
∞	12.50	4.17	2.50	1.25	0.62	0.50	0.25	0.17	0.13	0.08	0.06	0.05	0.00

When the number of males and females is unequal, the eggs and milt should be subdivided and paired before fertilization. When more than one male is to be used to fertilize an egg lot, milt should be collected from each male and thoroughly mixed before it is added to the eggs. This will reduce the probability that the majority of eggs are fertilized by the first male spawned.

Breeding Programs

A critical first step in the development of a breeding program is deciding on the program goals. Goals should be realistic and consistent with the eventual purpose of the fish. Obviously, the goals of an operation that raises salmon in sea cages differ from those of an operation that raises salmon for release into the wild to enhance a wild stock or repopulate a barren river. The first case can be likened to a beef feedlot and selection could be accomplished with genetic improvement methods similar to those used with domestic animals (Falconer 1981; Pirchner 1983). In domestic animals, such as poultry, swine, and cattle, national or State production associations have developed breeding goals and performance standards. Selection of superior individuals that produce progeny with superior performance has resulted in phenomenal advances in meat and milk production in the past 25 years. Fish culture is not as sophisticated as the culture of domestic animals, and the objectives of breeding programs in many fish hatcheries are not clearly defined.

Selection for performance in a particular environment is most effective when selection is conducted in that environment. Thus, selection in a hatchery for traits important to performance in the natural environment is ineffective because natural selection for population fitness in the natural environment is a stronger force than artificial selection. In many hatcheries, brood stocks are selected on the basis of subjective criteria that only the manager may fully appreciate. Experience teaches the manager to identify fish that perform well in the hatchery based on such characteristics as age, color, size, shape, or temperament. These characteristics influence the decision to spawn a particular fish; however, when a manager is asked specifically why one fish was chosen over another, it is often apparent that decisions were made with little true objectivity based on numeric criteria. This is not a criticism but merely an observation.

A breeding program for salmon destined for enhancement or recolonization must assume that the original population was, at one time, successful in the natural environment. A logical goal, therefore, is to attempt to duplicate the original population as accurately as possible. Information on the ratio of grilse to salmon, average size, migration patterns, timing of runs of smolts and adults, the proportion of precocious parr, and years at sea should be used as guidelines for the selection or identification of potential donor populations.

For enhancement and restoration programs, the genetic diversity and allele frequencies of the original stock should be maintained in the production fish. This concept differs from that of conventional selection programs that attempt to alter gene frequencies to improve particular traits.

In populations released into the wild, natural selection is a potent force that can increase the proportion of the particular gene combinations best adapted to the natural environment, but this will occur only if sufficient genetic diversity exists in the fish stocked. Wide genetic diversity is absolutely necessary to increase the probability that fish with superior gene combinations will be produced in sufficient numbers to survive the random mortality of the ocean fishery and return to spawn. This "shotgun" approach may be the best way to assure successful restoration.

An effective way to maintain genetic diversity is to spawn every fish that survives to maturity. If this is not practical because of the large number involved, then every effort should be made to use fish representing the entire population – that is, males and females maturing throughout the spawning season. Although it is common practice to spawn a portion of the adults and release the rest, this practice uses only a subset of the available successful gene combinations and could result in reduced genetic diversity if there is selection against early or late spawners. The hatchery manager should overcome the temptation to select brood stocks that conform to some perception of the ideal hatchery salmon or to spawn only fish that mature during a narrow period for the convenience of uniform spawning and incubation conditions. Unfortunately, the ideal hatchery fish is seldom the ideal wild fish, and poor selection decisions may result. Although hatchery efficiency undoubtedly improves, subsequent performance in the wild can deteriorate.

Brood stock should be randomly chosen from all returning adults, including grilse. If the selection is truly random, the proportion of grilse among the brood stock will be the same as that in the total population. Furthermore, males and females should be randomly paired for mating. Such a strategy involves identifying each adult return and pairing males and females by lottery. If all potential brood stocks do not mature simultaneously or are not available when spawning commences, then pairing should be among those available on each spawning day throughout the duration of the run. This ensures that gene combinations representative of the entire run are included in the progeny generation.

In many naturally spawning Atlantic salmon populations, precocious parr participate in spawning. As such, they must also be considered as successful

adults with respect to fitness and should not be excluded as brood stock. Because many precocious parr eventually become smolts, sea-run brood stock should contain some males that originally matured as parr. These fish would be identical to precocious parr in their genetic value. Thus, it may not be necessary to include mature male parr in matings produced in the hatchery.

One final consideration must be raised. In the wild, salmon of the same year class tend to become smolts over 2 to 4 years. However, in the interest of production efficiency, many hatcheries have adopted 1-year smolt programs in which fish that are still parr at 1 year are stocked along with the smolts. This practice sharply reduces the potential future genetic contribution from these slower growing fish, because these parr are usually placed in inappropriate nursery habitat. Although the long-term effects are largely unknown, in theory this practice would reduce genetic variation in the population. Therefore, it would be better, from a genetic standpoint, to retain such parr for release as 2-year smolts.

Recommendations

1. Establish breeding program goals as a first step in any selective breeding program. Goals should be realistic and consistent with the eventual use of the fish.
2. Minimize inbreeding to avoid a decrease in mean performance of traits associated with reproductive capacity and physiological efficiency. Most problems associated with inbreeding can be avoided if the minimum number of brood stocks used in each generation is maintained at or above 50 pairs (i.e., 50 males and 50 females). Single-pair mating—one male mated to one female—is strongly recommended.
3. Selection for performance traits will be most effective if it is conducted in the same environment where fish will be reared. Although not always possible, particularly when fish are produced in hatcheries for release into natural environments, every attempt should be made to minimize the length of time fish are maintained in hatcheries. Hatchery practices used in rearing the fish should mimic the natural environment to the extent possible. These steps will reduce the deleterious effects of domestication.
4. A primary goal of enhancement or restoration should be to conserve genetic variability of brood stock. Brood stock should be chosen randomly from the entire adult population, including both grilse and salmon. Parr should not be culled solely because they fail to become smolts in 1 year.

Chapter 3. New Technologies for Atlantic Salmon Breeding

by

Jon G. Stanley

U.S. Fish and Wildlife Service
National Fisheries Research Center– Great Lakes
1451 Green Road
Ann Arbor, MI 48105

The science of animal breeding has advanced rapidly over the past decade, especially in the field of fish breeding. Fish breeders used trial-and-error methods until the early 1970's when the theories of population genetics that developed in agricultural species began to be applied to fish populations. Fish breeders began to develop methodologies and technology uniquely applicable to the aquatic species. Many of these new technologies are still under development, whereas others are used routinely in applied fish-breeding programs. This chapter describes some of these new technologies and discusses the potential applications and benefits for Atlantic salmon breeding.

Genetic engineering in its broadest definition is the management and manipulation of gene pools to develop a biological product having practical use. This practical use may take many forms, ranging from development of populations with improved characteristics to microorganisms that produce purified enzyme products. One type of genetic engineering that has received considerable publicity is the splicing of chromosome pieces from one organism to another in such a way that the receiving organism is capable of manufacturing a chemical that would normally be produced only by the donor organism. Gene splicing, as this process is called, is not yet perfected for fishes, but studies are under way with various fish species. Foreign DNA injected into Atlantic salmon eggs can persist in the embryo for up to 14 weeks (McEvoy et al. 1988). The techniques of genetic engineering that involve identification and manipulations of single genes, multiple gene complexes, and chromosomes are discussed as they apply to Atlantic salmon by Stanley (1981) and Graham et al. (1985). Of special interest are techniques for the preservation of gametes and the production of monosex and sterile populations (Johnstone et al. 1978; Stoss et al. 1978).

Using traditional breeding methods of population genetics, the hatchery manager currently has the potential to engineer the kind of fish needed for most management applications. New methods for the production of monosex or sterile populations provide the opportunity to increase hatchery productivity and at the same time give the fishery manager increased control over the quality of the fish produced. These methods are valuable to managers in fisheries management and aquaculture because for the first time the manager can control reproduction in the environment being managed.

Genetic engineering techniques will not replace traditional breeding methods for the development of brood stock for hatchery production, but they do provide the manager with additional tools to control fish populations and to increase management efficiency. Fish populations can be produced to meet the specific management objectives of a particular fishery. Lots with an increased percentage of females can be produced to provide greater reproductive efficiency.

Monosex Populations

The production of populations containing only female fish in hatchery production lots has several applications (Table 3.1). Precocious males – early maturing fish that develop fully functional testes and display courting behavior as parr or grilse – would be eliminated in the all-female population. Males maturing as parr before migration or as grilse after 1 year at sea yield a smaller body weight when they return to the fishery. Females seldom mature as parr although a few may mature as grilse.

Grilse are a problem in many fisheries because these early returning fish, which are largely males, are much smaller and less desirable to the angler. The number of grilse in the population could be greatly reduced if the run consisted of mostly females. It would be feasible to use all females in a run if no natural reproduction occurred in the river system. The Union River in Maine is one such candidate for stocking only female fish.

All-female populations would allow the manager to increase egg production and simultaneously decrease

Table 3.1. *Benefits of monosex populations of Atlantic salmon.*

Sex condition	Potential application
All female	1. Eliminate precocious males
	2. Reduce grilse in returns
	3. Increase reproductive efficiency of population
All male	1. Use sex-reversed males to sire 100% female offspring
Sterility	1. Augmentation of natural runs without danger of dilution of gene pool through random hybridization of populations
	2. Production of trophy Atlantic salmon for sport fishery

the total number of brood stock that needs to be held in the hatchery by eliminating the male component from the brood stock. Males needed for milt production – including grilse and precocious parr – would be obtained from wild fish or other hatchery lots of the same stock. Runs used primarily for brood stock could be enriched with females by stocking some all-female lots. The need to sort fish at the trap for sex determination would be avoided, and the program would be assured of an adequate egg supply. In addition, the presence of surplus females on natural spawning areas would enhance reproductive potential because a single Atlantic salmon male will mate with several females. Enrichment of natural runs with additional females is not necessary in populations where annual sea returns yield adequate egg supplies to meet restocking needs for that river system but would be advantageous in situations where egg production does not meet stocking requirements. Migrating smolts from wild populations are often dominated by females (Jensen and Johnsen 1986).

Populations of Atlantic salmon containing only males would have little practical use in current management programs because of the expected increase in grilse frequency and absence of egg production in the population as adults. Conceivably, however, an all-male population could be used to enhance returns of stocks that are heavily fished on the high seas. The goal is to promote the percentage of males that return to the river as grilse. Because they are in the ocean for a shorter time and are smaller, more would be expected to escape the high seas fishery and, therefore, yield a higher rate of return. The use of male monosex populations may also be beneficial for fisheries where runs have little potential for natural reproduction.

The primary use of genetically engineered male fish, however, is in the production of sex-reversed male gametes necessary to sire all-female lots. The mechanism of sex determination in most fish species involves the inheritance of sex chromosomes that determine genetic sex. Sex chromosomes direct the production of specific hormones that determine functional sex. Most fish species, including Atlantic salmon, express the X–Y system of genetic sex determination whereby the inheritance of two X-chromosomes yields females and the inheritance of an X- and a Y-chromosome produces males. Females are called the homogametic sex, because only one kind of gamete is produced. Males are referred to as the heterogametic sex and can produce gametes that contain either X- or Y-chromosomes. The genetic sex inherited and the functional sex expressed by the individual can be different if sex-reversal techniques are applied at the appropriate time. Genetic sex is determined at fertilization by the particular set of chromosomes inherited by the individual. Functional sex is not determined until later in the development stage when genes on the sex chromosomes are activated to produce specific hormones that control sexual differentiation.

Sex-reversal procedures have been successfully applied to both sexes in Atlantic salmon. The procedure involves exposing the fish to appropriate hormones during sexual differentiation in sufficient concentrations to mask naturally produced hormones, thereby causing all individuals to develop into the desired sex (Yamamoto 1969; Schreck 1974). Early efforts to reverse sex in fish species were inconsistent, largely because treatments began after the sex differentiation process had already begun. Johnstone et al. (1978) found that the outcome of hormone treatment depended on precise timing. Females were produced in rainbow trout when eggs were immersed in estrogen followed by feeding estrogen to the fry. Hermaphrodites developed when the treatment began after the fry had started feeding. Sex-reversal from female to male using methyltestosterone was not as successful, however, because some individuals remained female and some became hermaphrodites. Johnstone et al. (1978) found sex-reversal was easier in Atlantic salmon than in rainbow trout. All females were produced with estrogen treatment, and all males were produced with methyltestosterone, regardless of whether treatment began before or after hatching. These salmon were not reared to maturity, so it is possible that the males could have been sterile, because sex-reversed rainbow trout

males do not have a sperm duct. Fish could not be spawned externally but had to be killed and the gonads removed to obtain sperm. A positive aspect of this finding is that the sex-reversed females (functional males) can be easily identified without progeny testing for use in matings to produce the monosex female populations.

Sex reversal in Atlantic salmon has been successful when hormones were mixed with the daily feed ration (Johnstone et al. 1978). The recommended procedure to sex-reverse male to female is to feed fry a diet containing estradiol at 30 mg/kg of diet for 80 days starting at first feeding. The feed is prepared by dissolving 30 mg of 17(beta)-estradiol in 1 L of 95% ethanol and mixing thoroughly with 1 kg of feed. The feed is then dried – in either oven or air – to remove the alcohol. It is generally recommended that fresh feed be prepared each week if it is to be stored at room temperatures; it can be prepared less frequently if storage is at temperatures of 0°C (32°F) or lower. The procedure for sex reversal from female to male involves feeding a diet containing an androgen. The recommended androgen is 17(alpha)-methyltestosterone, and the treatment level is 5 mg/kg of diet. This hormone can be incorporated into the diet by dissolving in ethanol, mixing, and drying as described previously.

Sex-reversal techniques can produce monosex fish populations; however, the procedure cannot be used directly on fish used for stocking or human consumption, because the inducing agents are not registered for fishery use. This problem can be avoided if progeny from sex-reversed fish are used for the stocking program, rather than using the sex-reversed individuals. The progeny generation is produced by mating known sex-reversed fish with normal individuals (Fig. 3.1). To produce a female monosex population in a species with female homogamety (female XX and male XY), such as the Atlantic salmon, the first step is to treat fry with methyltestosterone to produce a male sex-reversed lot. When these fish reach sexual maturity, the sex-reversed fish (XX males) can be identified as those without a sperm duct. Fish capable of expressing milt externally are normal XY males and can be discarded. Milt is obtained from the sex-reversed XX males by removal and maceration of the gonads. After collection, milt can be used directly on eggs or extended in saline solution before use.

A second method for producing monosex female Atlantic salmon uses artificial gynogenesis, a type of parthenogenesis first described for the brown trout (*Salmo trutta*; Oppermann 1913). Gynogenesis is the development of the egg caused by penetration of a sperm, but without fertilization; in other words, sperm chromosomes do not contribute to the development of the embryo. Artificial gynogenesis is induced by using

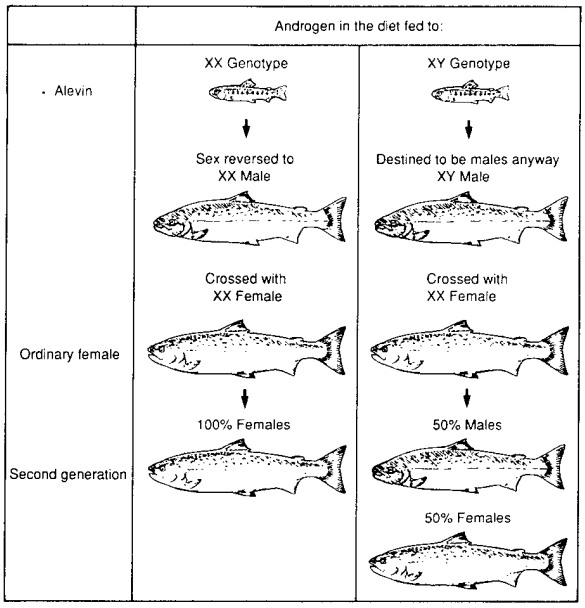

Fig. 3.1. Scheme for breeding sex-reversed XX-males to produce all-female progeny. The rationale is to produce XX-males, which are then bred to ordinary XX-females to produce all-female XX-progeny.

irradiated sperm. The irradiation completely denatures the paternal chromosomes without destroying the flagella or microtubules, which are the sperm components responsible for activating egg development. The resulting embryo is haploid with a single set of maternal chromosomes. Haploid embryos have a limited ability for growth, and none survive to hatching.

Gynogenesis can be useful, however, if the haploid embryo can be made diploid by inducing retention of a second set of maternal chromosomes. A second chromosome set will spontaneously remain in a small percentage of eggs. Rate of retention of the second set can be artificially increased by thermal shock of the egg shortly after fertilization. Although cold shocks are effective in some fish (Stanley 1981; Donaldson and Hunter 1982), they have little effect on the Atlantic salmon (Lincoln et al. 1974; Allen and Stanley 1981). In rainbow trout, immersion of the egg in hot water increases the percentage of embryos that retain extra chromosome sets. Rainbow trout eggs treated for 10 min with water heated to 28°C (82°F) – beginning at fertilization – gave best results (Chourrout et al. 1980). Also, rainbow trout produced from eggs shocked at 27–28°C (81–82°F) for 10–15 min – starting 40 min after fertilization – all had extra chromosome sets (Lincoln and Scott 1983). Higher temperatures for brief periods worked – for example, 36°C (97°F) for 1 min (Thorgaard et al. 1981) – but yielded lower survival

rates of developing embryos. Brown trout eggs shocked at 32°C (90°F) produced all triploid fry (Arai and Wilkins 1987).

Either X-ray or ultraviolet irradiation denatures chromosomes in sperm. Ultraviolet is recommended because it is safer, and the equipment is less expensive. The procedure uses milt mixed with 4 times its volume of cold Hanks' balanced salt solution. This mixture is placed in an uncovered petri dish 15 cm below the UV bulbs of a sterilization apparatus. The mixture is shaken during a 5-min exposure to the irradiation (dose: 6 mW/cm^2, wavelength 254 nm). The center of the UV bulb should be used as the focal point for the treatment because the ends of the bulbs produce less irradiation. Safety procedures require that goggles and gloves be worn to protect the eyes and skin. In addition, the UV apparatus should be shielded from potential observers for their protection.

If gynogenetic Atlantic salmon are to be useful there must be assurance that growth or performance is not depressed by the increased homozygosity that may accompany restoration of the diploid condition. Because both sets of chromosomes are derived from the mother, the increased level of homogeneity or inbreeding that may result from restoration of diploidy by this method will vary from 25% (full-sib mating) to 100% (selfing). Atlantic salmon produced by gynogenesis may express reduced survival and growth rates relative to bisexual fish (Purdom and Lincoln 1973; Cherfas 1975).

Sterile Fish Populations

The ability to produce many sterile fish for direct stocking would provide the fishery manager several new options. Potential dilution of unique gene pools resulting from interbreeding with hatchery stockings could be prevented if hatchery-stocked fish were sterile. Hatchery smolts could thus be stocked in a stream with a depleted natural population to provide angling for the fishery without danger of diluting the existing gene pool when the stocked fish reached sexual maturity. Sterile fish could also serve as a buffer against fishing pressure and predation.

Sterile fish could contribute to a trophy fishery. Because sterile fish expend less energy in the production of sex products, this productivity can be transferred into increased flesh production. These fish would also express a continuous growth rate without the annual shift from growth to reproductive phases. One management approach to provide these larger fish might be to stock a percentage of sterile fish, with the expectation that some would survive to become trophies. A large size might also be attained because of an extended life span. In theory, fish that do not reproduce should have lower mortality and continue growing throughout the life cycle.

Salmon that overwinter in fresh water could provide a significant spring fishery. Ordinary black salmon or kelts are in poor condition to survive overwintering, but sterile fish may be in much better condition and would be more desirable to the angler. Rather than being a primary management objective, this increased fish quality in the sterile population would be a fringe benefit.

The gain to management from the use of sterile Atlantic salmon populations is unproven, because little research on the field performance of sterile fish has been completed. In a laboratory study of triploid Atlantic salmon, the oxygen-carrying capacity of the blood was reduced (Graham et al. 1985); this reduction could compromise the ability of the fish to survive in the wild. Questions remaining are whether sterile Atlantic salmon will return to the river of release and when. A return migration might be expected, because Atlantic salmon initiate migration to fresh water before they reach sexual maturity—migration is not triggered by the high levels of hormones secreted during courtship and spawning. Studies of sterile Pacific salmon show that they return, but return may be delayed—in one experiment with chinook salmon (*Oncorhynchus tshawytscha*) the fish did not return until they were 6 years old (Hershberger et al. 1978).

Another method for obtaining sterile populations is to produce triploids. The three chromosome sets in these individuals produce sterility because homologous chromosomes are unable to synapse during gametogenesis and therefore yield unbalanced, non-functional gametes. The same temperature-shocking procedure described in conjunction with gynogenesis to cause retention of an extra set of chromosomes is also used to produce triploids (Fig. 3.2). The difference is that triploid rather than diploid individuals are produced when normal untreated milt is used because three chromosome sets are incorporated in the embryo—one from the egg, one from the sperm, and one from the polar body.

Triploidy was first observed in Atlantic salmon in a hybrid mating with brown trout when the egg was chilled after fertilization (Svardson 1945). Triploids induced from pure Atlantic salmon have been obtained by treating newly fertilized eggs with the chemical cytochalasin B (Refstie et al. 1977; Allen and Stanley 1979, 1981). These Atlantic salmon did not have all triploid cells in their tissues; instead they were mosaics composed of diploid, triploid, and higher-ploidy cells. Many of the mosaic cells disappeared as the fish matured after stocking into a Maine lake, so that after 3 years only diploid cells remained (Allen and Stanley, unpublished observations).

Triploidy in Atlantic salmon is not yet fully understood. Additional work is needed to refine the

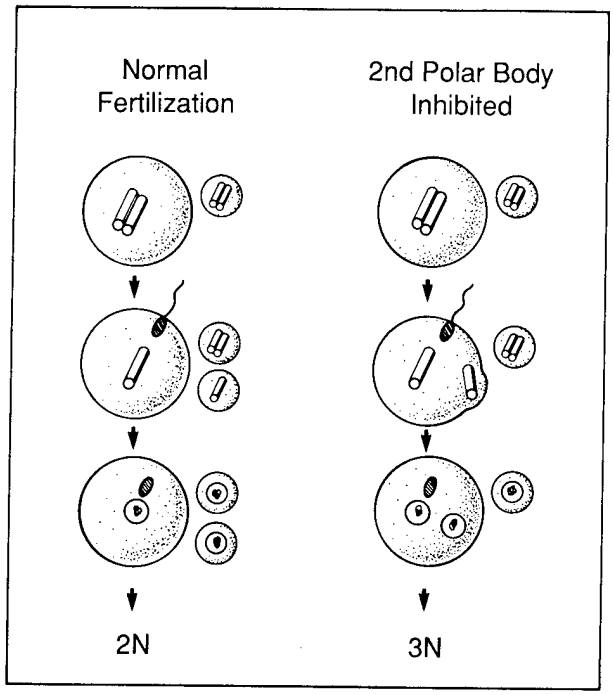

Fig. 3.2. Mechanism for producing triploidy in fishes. In normal fertilization of the egg (represented by the larger sphere) the first polar body (represented by the smaller sphere) is expelled before fertilization, carrying with it two sets of superfluous chromosomes (represented by the two bars). After fertilization another set of chromosomes is expelled in the second polar body. Triploidy is induced (on the right of the diagram) when expulsion of the second polar body is inhibited. The resulting offspring has two sets of chromosomes from the female and one from the male.

techniques for producing it, especially the optimum timing and duration of temperature shocks and hydrostatic pressure. A second method for producing triploids is to develop tetraploid lines that would then be crossed with normal diploid fish to produce a 100% pure triploid. Significant progress has been made in sterilizing male Atlantic salmon through autoimmunity of spermatozoa (Secombes et al. 1987).

Kelt Rejuvenation

Atlantic salmon, unlike Pacific salmon (*Oncorhynchus* spp.), do not die after spawning. Under natural conditions, kelts spend the winter in fresh water after spawning and return to the sea in spring. Salmon resume feeding at sea after about 12 months of fasting and a loss of almost half their body weight. Only about 10% of the kelts survive to return for a second spawning.

Atlantic salmon brought into the hatchery for spawning can be retained afterwards and the kelts reconditioned. The kelts are placed in tanks with subdued light and coaxed to begin feeding. At first, food is presented to each fish individually, but they soon learn to take food thrown into the tank. Kelts can be rejuvenated effectively in fresh water or salt water. Once the fish are enticed to resume feeding, the survival rate is about 75%. The fish should be moved to outdoor tanks under natural light conditions in late March, as day length increases after the vernal equinox. Most of the rejuvenated fish will become sexually mature for a second time, some at the end of the first year and some after 2 years in the hatchery.

The success of kelt reconditioning is heavily dependent on proper nutrition. Vitamin and mineral deficiencies lead to poor egg quality and subsequent high mortality of embryos. The kelt diet used at the Berkshire (Massachusetts) Trout Hatchery consisted of 25% liver, 25% fish, 50% salmon starter, and a vitamin and mineral pack. When nutrition is adequate, the reproductive performance of kelts (egg size, egg number, and egg hatchability) is comparable to that of sea-run fish. Progeny from rejuvenated kelts have an ocean return rate similar to progeny from maiden brood fish.

A number of genetic implications are inherent in a kelt rejuvenation program. One benefit is that maximum use is made of fish returning from the sea. In a restoration program where the original stocks are extinct and only a few adults are expected to return during the early phases of restoration, effective use of available brood stock is especially important. The use of rejuvenated kelts allows the successful sea-run returns to produce more offspring over 1 to 4 years, thereby enhancing the effects of natural selection in the ocean environment. Using kelts derived from a particular river system as the principal egg source for restocking also permits more rapid progress toward the development of strains genetically adapted to that river system. I recommend that, if possible, kelts selected for a reconditioning program be animals that have spent their life cycle – both the freshwater and seawater phases – in the wild. Genetic traits favored by natural selection in both environments could thus be enhanced by the kelt reconditioning program.

The negative aspect of a rejuvenation program is that the founder population may be very small, thus the restoration effort begins with a limited gene pool. The danger is that if progeny from reconditioned kelts return in greater numbers than smolts transplanted from other sources, the advantages of reconditioning could be lost in two to three generations due to inbreeding. The same problem occurs any time a limited number of brood fish are used. Using few brood fish each generation without periodic introductions of new genetic material leads to inbreeding depression. I advise against reconditioning captive brood stocks that have spent their entire life in the hatchery. This practice would lead to the

enhancement of traits favored by adaptation to the artificial environment, or domestication. However, this approach would be a good procedure for private growers interested in developing strains for commercial production in sea cages. I recommend that kelt rejuvenation be used in restoration programs.

Advancement of Gonadal Maturation

Atlantic salmon captured on returning to their home rivers are often held 3–6 months before spawning. During this period, sexual maturation progresses to spawning in November, when temperatures would be favorable for spawning if the fish had remained in the natural environment. This maturation cycle is timed so that spawning does not take place prematurely, when water temperatures are too high for egg incubation, or too late, when water temperatures are too low for fertilization.

The process of sexual maturation depends on three variables: time, water temperature, and photoperiod. Time is required for gonadal growth, during which nutrients are mobilized from body reserves and deposited in the gonads. In Atlantic salmon, declining water temperatures and decreasing daylight may promote gamete maturation and ovulation. Photoperiod and temperature elicit the hormonal changes that stimulate this gonadal maturation. By artificially advancing these seasonal changes, hormones are secreted earlier and the hatchery manager obtains mature brood fish earlier in the season. In other salmonids, photoperiod is more important than temperature in controlling sexual maturity (Carlson and Hale 1973; Kunesh et al. 1974). In pink salmon (*Oncorhynchus gorbuscha*), sexual maturation can be induced in the first year of life by using heated water (MacKinnon and Donaldson 1976). Light conditions are easy to control if brood fish are held indoors. A controlling device (locally available and inexpensive) is connected to the lighting system to automatically turn lights on and off. Photoperiod controls should provide for phasing of the light to simulate twilight and to assure that fish are not disturbed by sudden changes in light intensity. Temperature can be controlled by artificially cooling the water, but this may be expensive and less effective than photoperiod control. However, it is important that brood fish be in cool water before spawning to ensure good egg quality.

The fish could be injected with hormones to artificially stimulate maturation rather than manipulated by environmental factors. Purified pituitary gonadotropin is available from commercial sources; it induced ovulation when injected into pink salmon (Donaldson et al. 1972) or coho salmon (*Oncorhynchus kisutch*; Sower et al. 1982). Injections were given 3 times per week at a rate of 1 mg/kg of body weight of gonadotropin dissolved in 0.5 mL of fish saline. This approach for advancing sexual maturation produced good results but required frequent handling of brood fish. Luteinizing hormone-releasing hormone in silastic implants reduced handling and was effective in advancing maturation of Atlantic salmon (Crim et al. 1986).

I recommend the use of modified photoperiods to manipulate the timing of sexual maturation. Salmon gonadotropin can be used to induce final maturation if the fish do not mature according to the new schedule. Water should be cooled gradually over the final 2 weeks to temperatures that duplicate those experienced during spawning in nature. Cooled water may also have to be used during early incubation of eggs. It should be possible to advance spawning by about 1 month with little or no decrease in egg quality. The advantage of advanced spawning is earlier hatching, which would yield increased frequencies of 1-year smolt and, when used in combination with heated water after eggs reach the eyed stage, could result in 100% 1-year smolts. Hormone treatment can also serve to shorten the spawning season by synchronization of spawning time in Atlantic salmon (Crim et al. 1986).

Storage of Gametes

Atlantic salmon may not mature at a time convenient for the breeder. It would be desirable if sperm and eggs could be stored so that fertilization and incubation could begin at a particular time. Also, mates could be selected more precisely if gametes could be stored and combined after breeding schemes were planned. This is important because the breeder cannot always predict when individuals of each sex will become ripe. When gametes are stored, breeding pairs can be chosen on a basis other than the fact that both were ripe at the same time.

Storage of gametes would be especially valuable in a breeding program that involved mating particular genotypes identified by isozyme analysis. Where individuals must be killed to obtain body tissue for analysis, gametes could be collected and stored before the potential parents were sacrificed for isozyme analysis. Desired matings, to produce particular allelic combinations in offspring, would be made by using gametes from the individuals identified.

Gametes may be stored short-term or long-term. Short-term storage involves maintaining the material in a liquid state, whereas long-term storage requires freezing. Both can be achieved, although at present reliability cannot be assured.

Short-term cold storage was very successful for rainbow trout sperm (Stoss et al. 1978). Sperm retained the ability to fertilize eggs for 23 days when stored undiluted at –2°C (28°F). The atmosphere above the storage tubes was pure oxygen that was changed daily.

Agitation of the storage tubes was not beneficial and resulted in reduced sperm viability. Short-term gamete storage techniques are available that allow fish breeders to accomplish several things. Milt can be taken from mature males and held either individually or in pools until needed. Also, milt and unfertilized eggs can be shipped to other stations for use in crossbreeding and hybridization programs.

Atlantic salmon gametes have been frozen for long-term storage. Freezing is more effective for sperm, but eggs and embryos can also be frozen (Zell 1978). Atlantic salmon sperm retains its effectiveness after thawing when frozen at –8°C (18°F). Milt is prepared for freezing by first mixing it with an equal volume of Poulik's solution (85 μmol glycine, 4.9 g NaCl, 2.3 g KCl, and 100 g of polyvinylpyrrolidone, diluted to 1 L with distilled water and adjusted to pH 8.8 with HCl or NaOH). The milt mixture is frozen in plastic straws so that rapid cooling occurs at the rate of about –5°C/min (–9°F/min). Sperm can be kept frozen for several months.

Zell (1978) also froze unfertilized eggs, newly fertilized eggs, and eyed eggs and obtained hatching after thawing. The highest success rate was obtained when zygotes were frozen at –5 to –12°C (10 to 23°F) in Hanks' solution. The thawing rate also affects survival; unfertilized eggs and zygotes survived best when thawed slowly and eyed embryos when thawed rapidly.

The techniques for gamete storage offer Atlantic salmon breeders a versatile method for managing the breeding system, independent of the time of final sexual maturation. Fewer fish have to be handled during the busy egg-taking period, because milt can be taken beforehand and stored for use as needed. When gamete storage is available, breeders may be tempted to use milt from a limited number of males considered to be superior for multiple fertilizations. This practice should be avoided because of the danger that the number of males used as parents could become very small, leading to high rates of inbreeding. In addition, there is a real question about the ability of breeders to identify truly superior fish based on phenotype or attained size.

Population Identification

Stocks are the units of management in Atlantic salmon that need to be identified and characterized. Managers can use several genetic characteristics to identify the stock to which an individual belongs. Ideally, the identifying traits should be adaptive, with different phenotypes associated with specific performance characteristics. It would be convenient if the fish could be examined to determine the specific characteristics that allowed it to live in a specific local environment. However, only a few such specific adaptive traits are known for Atlantic salmon – fin length, which is an adaptation for living in fast currents (Riddell et al. 1981), and direction of orientation of fry as they disperse from the redd.

There are many ways to identify specific stocks based on characteristics measured indirectly. Morphological traits, such as body dimensions, and meristic traits, such as scale counts, are two types of traits for distinguishing Atlantic salmon stocks (MacCrimmon and Claytor 1985). Differences between stocks may be caused by either natural selection, where the traits differ genetically due to adaptation to different habitats, or physiological changes, where the traits are not genetically modified by the environment. An example of the latter situation is number of vertebrae as a function of temperature – fish embryos developed at low temperatures have more vertebrae than those developed at high temperatures. Another possibility is that a characteristic may have a genetic basis but it is not adaptive.

Stock differences are detected by measuring many traits on several specimens of each stock. Measurements might include counts of fin rays, body scales, pigment spots per scale, gill rakers, pyloric caeca, and body measurements of fin length, depth of body, length of body, head length, and eye width. The data are then analyzed by a powerful statistical procedure such as Discriminant Function Analysis. This procedure is available as a standard software package on most mainframe computers. With Discriminant Function Analysis, stocks can be distinguished from each other; however, it will not determine if a specific fish is a member of a particular stock.

Scale shape has been used as a method of stock identification. Scales are removed, magnified about 20×, and photocopied on a microfilm printer. The shape of the scale perimeter is traced on a digitizer, and the shape is analyzed by Discriminant Function Analysis. The principal advantage of this method is the speed and simplicity of the sampling. In addition, the sampling does not require that the fish be killed.

Fish in their natural environment absorb minerals, which are deposited in bony tissues. Different geographical areas have different mineral compositions; thus, fish may be labeled naturally with these elements. Analysis by X-ray spectrometry provides a profile of the elemental composition of the bones. A fish can be marked with an unusual element, such as the rare earth element terbium (Tb), as a permanent label. This methodology is not useful for stock identification in Atlantic salmon, but could be used to answer specific questions. An adult brood fish injected with a mineral solution incorporates the mineral into the eggs. The resulting fry carry the mineral. This procedure could be used experimentally to determine if hatchery-produced fish contributed to a spawning population. Returning adults could be

captured from the run and those originating from the hatchery injected with terbium. Analysis of fry the following spring would reveal the contribution of the injected fish to the fry produced.

Electrophoresis has been the most widely used method for identifying fish stocks. The procedure separates different proteins in an electric field. A protein solution is developed from tissue samples and placed in a medium (usually a gel of potato starch) and an electric current is applied. After the proteins are separated, a stain is applied that permits a specific protein to be seen. Stains are specific agents that change colors when a substrate is added to the protein. The different proteins or enzymes are the products of single genes and have a genetic basis. Just as any genetic trait may take different forms, these enzymes occur as variants. The different forms can be distinguished by differences in migration in the starch gel. Electrophoresis is done on either fresh or frozen tissue from muscle, brain, heart, eye, or pyloric caeca after they are squashed and extracted in water. Blood can be used for some enzymes.

Electrophoretic variation is a practical tool because the genotypes are retained throughout life, are not modified by the environment, and are inherited in offspring in a predictable manner. These variations can be used to determine stock differences and also to document specific matings. In Atlantic salmon, electrophoresis is most useful in separating major groupings. North American stocks differ from European stocks. Two stocks have been identified in the British Isles. The Baltic salmon is different from other European Atlantic salmon. By analyzing numerous traits, major stocks of Atlantic salmon can be distinguished. However, the method is not powerful enough to distinguish between local stocks within a river system or to assign an individual fish to a particular stock based on biochemical traits.

Electrophoretic procedures have many other applications. Enzyme differences between species identified by electrophoresis can be used to positively identify a species. This is important in law enforcement where species identification must be proven for fish alleged to have been poached—for example, were the fish Atlantic salmon or imported Pacific salmon? Likewise, hybrids between species are easily detected. A recent study in England found that hybrids between Atlantic salmon and brown trout were common in the wild. In New Brunswick, salmon in the Big Salmon River were thought to be hybrids with brown trout, based on their life history and behavior. Beland et al. (1981) showed that enzymes were predominately those of Atlantic salmon, with one gene that suggested some past interbreeding with brown trout.

Electrophoresis can be used to assess the degree of inbreeding by comparison of the current level of homozygosity with that of the base population. Because the background homozygosity in Atlantic salmon is very high, detection of inbreeding with this method is difficult.

Genetic similarity can be determined by using electrophoresis. Populations that have the same isozyme frequencies are presumed to interbreed or to have diverged from a common ancestral population in the not-too-distant past. Findings from isozyme data indicate that Atlantic salmon populations are quite similar over wide geographical areas; however, local populations exist. Salmon adapt to local conditions much more rapidly than isozyme mutation or changes due to genetic drift. Therefore, genetic diversity estimates based on electrophoretic data from different populations probably underestimate the real genetic differences between Atlantic salmon stocks.

Recommendations

1. Develop techniques for application at the hatchery level to produce early maturation of brood stock so that egg taking can be advanced and provide a longer growing season.
2. Develop techniques for application at the hatchery level to establish sex-reversed XX male brood stocks for breeding all-female smolt production lots. Monosex female production lots will enhance reproductive efficiency in small-scale, moderately effective restoration programs.
3. Develop techniques for application at the hatchery level to collect and store milt at brood stock hatcheries. Milt storage procedures, both short-term and long-term, will reduce the need for brood stock handling and improve effectiveness of random mating goals in hatchery and wild stocks.

Chapter 4. Methods for Developing New Stocks of Atlantic Salmon

by

John D. McIntyre

U.S. Fish and Wildlife Service
National Fisheries Research Center – Seattle
Building 204, Naval Station
Seattle, WA 98115

The productivity of many streams in New England for Atlantic salmon is being restored through abatement of water pollution and removal of barriers to migrating fish. As a result, interest is increasing in reestablishment of natural spawning runs of Atlantic salmon in some of these streams. I describe methods intended to help develop and maintain "new" stocks of these fish.

Characteristics of Stocks

Ricker (1972) wrote that a stock is "the fish spawning in a particular lake or stream (or portion of it) at a particular season, which fish to a substantial degree do not interbreed with any group spawning in a different place, or in the same place at a different season." Ricker went on to say that "substantial degree" is open to interpretation and is not meant to exclude all exchange of genetic material between stocks, but only to limit the exchange to the point that distinctive stock characters that adapt individuals to specific local habitats will be maintained. The terms strain and stock are used interchangeably throughout this chapter to refer to different domestic and natural populations with strain being used more in reference to domestic populations and stock to wild populations (see glossary for specific definitions). There are at least 350 stocks of wild Atlantic salmon (Chadwick 1985).

Genetic differences among stocks can be demonstrated by rearing representatives from several stocks under uniform environmental conditions and measuring the variation that persists. Variation in growth rate and age at maturity persisted among Atlantic salmon from 16 locations after they all were reared in a common environment at a single location (Naevdal 1981). Probably the most compelling evidence that many differences among stocks reflect genetic adaptations is the low survival of stocks transported to remote locations relative to survival in their native environment (Ritter 1975). Survival rate tends to decline as the distance increases between the water in which a stock is relocated and its home stream (Reisenbichler 1988).

Genetic variation exists not only among stocks but among individuals within a stock, and this variation aids the persistence of the stock in the face of changing environmental conditions. Harsh environmental conditions, such as sporadic epizootics, result in a survival preference for certain phenotypes (e.g., those bestowing disease tolerance). Without such phenotypes (i.e., without sufficient genetic variation) the stock might not keep pace with changing environmental conditions and be lost. Genetic diversity can be created by crossbreeding of stocks, but the diversity needed in a healthy stock is that which results from natural selection in their native environment and not the diversity created by extensive outbreeding.

Low levels of outbreeding can be important under natural conditions, because the amount of habitat available to some stocks is not sufficient to maintain the number of fish needed to prevent excessively high rates of inbreeding. The deleterious effects of inbreeding can be severe in small, reproductively isolated stocks, resulting in unstable stock size and an increased probability of extinction. Outbreeding naturally occurs through occasional matings with strays from other populations and generally precludes the ill effects that result from inbreeding. Extensive outbreeding, however, may disrupt a coadapted genome and cause a sharp decline in fitness (Shields 1982).

New Stock Development

Neither the environmental factors that cause divergence between stocks nor the specific adaptations within a stock to accommodate these factors are sufficiently understood to predict the specific characteristics required in a population for successful colonization of a new location. It can be said, however, that development of a numerically stable, naturally reproducing stock depends on the extent to which

managers can simulate the natural processes that prevent high rates of inbreeding, while at the same time ensuring that the average fitness of fish in the stock increases.

The quality of the fish depends on their adaptedness for the environment at the restoration site. The best source of fish must be identified largely by trial and error, but in general, fish from stocks located near the restoration site, measured along water routes, should be chosen. Even fish from nearby waters may survive poorly, but average fitness should increase in subsequent generations if successful spawning occurs.

Existing stocks of Atlantic salmon in New England are an obvious source for development of new stocks, even though most of these available stocks depend on supplemental stocking from hatcheries and are composed of fish that are themselves in the process of adapting to their new environment. Fish from the available stocks can provide the best source of fish already adapted for survival in similar nearby streams. As the run size increases, fish can be expected to stray to nearby natural areas for spawning and rearing. Some of the adult fish produced from natural spawning should be captured and used in the local hatchery to produce the next generation, thereby avoiding the concentration by natural selection of gene combinations that adapt fish to the environment provided in the hatchery. Managers should ensure that enough parents are used each generation to prevent the gene pool from being restricted by inbreeding and that artificial selection does not occur. Fish from remote locations, or from other projects at the hatchery, should not be used as supplemental brood fish except in the early stages of a program when the number of wild brood fish is low.

The obvious first step in developing a new stock of naturally spawning fish is to produce a run of adults to the restoration site. The most effective approach probably is to release what may seem to be an excessively large number of smolts at the restoration site to help compensate for deficiencies in their fitness and to enhance the probability that a run of adults will be produced. Smolts should be released so that they remain in the stream only long enough to imprint before migrating to the sea. Smolts should be released each year for at least 3–5 years after the restoration program begins to ensure returning year classes throughout the generation interval.

Smolts are recommended for release because of the difficulty in estimating the number of fry needed to produce a population matching the available habitat productivity and the unknown fitness of the fish to be released. If smolts are released, but no adults return to the site, conditions other than habitat quality at the restoration site probably are the cause. A similar result with fry releases would not distinguish between poor conditions at the restoration site and poor fry quality as the cause for failure. If no spawning adults return from releases of smolts, then the smolts had low fitness or high mortality unrelated to fitness. It is important to determine which of these explanations is correct if appropriate action is to be taken.

It is not enough to just get adult fish back to an area. Conditions must exist for successful reproduction so that these adults produce the same number or more adult offspring. Mortality caused by water development projects and fishing can hamper stock development efforts even when the productivity of rearing areas has been fully restored and fish of high quality are available for stocking. The significance of this mortality can be seen by assuming that a representative female from a highly adapted stock, spawning in productive habitat, may produce about 100 smolts resulting in 5 adult offspring. This stock can withstand harvest of 60% of the adults produced and still replace itself. Removal of 50% of the smolts produced, either by fishing or by killing them at water development projects, reduces the allowable harvest fraction to 20% of the adults produced. Clearly, projects undertaken to restore naturally spawning stocks must include measures to control mortality in the fish produced so that replacement spawning can occur.

Managers attempting to develop new stocks must determine if fishing is too intense to warrant an attempt at restoration, and they must continue to monitor fishing after the project has started. Survival may be inadequate for restoration of the naturally spawning stock; however, it may be possible to increase survival to maturity by limiting total catch to present levels and reducing the harvest fraction by increasing the supply of adults. If each spawning pair in a new stock produces only three adult offspring (recruits), expansion of the stock cannot be expected if the harvest fraction is as much as 33%. Assuming that the present harvest fraction is 70% and the goal for the new stock is 20%, the population of recruits must be increased 3.5-fold while keeping present total catch constant if the harvest fraction is to be reduced from 70% to 20%. Given this (perhaps extreme) example, hatchery effectiveness, and possibly hatchery capacity, will have to be greatly increased to produce these adults. As the stock becomes better adapted and appropriate escapement levels are approached, restrictions on catch can be liberalized in annual increments consistent with any increasing harvestable surplus.

Recommendations

1. Release smolts produced in hatcheries to stimulate development of new stocks of Atlantic salmon, because many more smolts can be released into a limited area than could be produced from fry releases in the same

area. Stocking fry rather than smolts will yield the benefits of natural selection more quickly, but the number of smolts they produce will be limited by the productivity of the available habitat. Some managers may find it possible to release both fry and smolts.

2. Smolts should be released so that they remain in the stream only long enough to imprint to the restoration site before they migrate to the ocean.

3. Stocks from nearby waters should be used as sources of fish to develop new stocks unless the environment of the nearby stock is obviously different from the environment at the restoration site. Transfer of fish from remote sites increases the possibility that their survival will be low, and crossbreeding of stocks may disrupt coadapted gene complexes. These possibilities should be evaluated as a part of an experimental program conducted in association with efforts to develop new stocks of Atlantic salmon.

4. Mortality must be limited to permit reproductive rates that will let the size of a developing stock grow to a level that can be supported by available habitat and provide a harvestable surplus.

Chapter 5. Atlantic Salmon Handling and Spawning Protocol

by

Michael Hendrix

U.S. Fish and Wildlife Service
Northeast Fishery Center
P.O. Box 75
Lamar, PA 16848

The native populations of Atlantic salmon remaining in New England streams have an inherently low survival rate due to high natural mortality during early life stages in nursery areas and high fishing mortality while on the feeding grounds in Davis Strait. Because their natural productivity is low, Atlantic salmon should be protected to enhance their biotic potential until the stocks can be rebuilt. The most practical method for promoting increased productivity is to protect the juvenile stages, which have the highest mortality, by bringing these early life history stages into the hatchery.

The cornerstone of the New England salmon restoration program is a multimillion-dollar hatchery system. Five hatcheries and a rearing station hold brood stock and produce large numbers of fry, parr, and smolts. Brood fish are captured as they return to several rivers and are held in the hatchery until they mature. Other brood stock fish are held captive throughout life. After spawning, eggs are distributed to appropriate rearing facilities, and a combination of fry, parr, and smolts are subsequently planted in restoration rivers. The challenge facing managers of Atlantic salmon is to combine state-of-the-art culture techniques with sound genetic principles.

The restoration of Atlantic salmon depends on a hatchery program that annually uses about 1,500 adult brood fish to produce more than 4 million fish for stocking. Hatchery procedures are needed that minimize the risk of disasters and result in rapid progress toward achieving restoration goals. In this chapter, I describe handling and spawning protocols that have proven to be good hatchery practices.

Handling Protocol

Sea-run Atlantic salmon adults are trapped in rivers and transported to hatcheries in tank trucks. Water in the transport tank is aerated and, if necessary, iced to maintain temperatures at 16°C (60°F) or lower. On entering the hatchery, fish may be injected with an antibiotic to help them withstand handling stress and prevent possible disease outbreaks caused by pathogenic bacteria. Injections are especially important if furunculosis and enteric redmouth disease are present. The antibiotic protects the fish for only a few days but enables fish to recover from handling stress so their own defense mechanisms are able to combat disease organisms. Longer-lasting vaccines or a combination of vaccine and antibiotic are not recommended by fish pathologists, because the vaccine is thought to overload the immune system already compromised by stress. If a vaccine is given, it should be injected well after initial handling stress has passed. Injections must be given intraperitoneally, exercising care to avoid damaging internal organs with the needle or by discharge in the musculature. I recommend that fish be anesthetized before injection.

Sea-run Atlantic salmon are especially sensitive to handling-induced fungal infections. Within 1 to 2 weeks after transfer to holding pools, many fish succumb to fungal infection unless the pools are treated with a fungicide. I recommend that fungicides be administered at least 2 times per week for the first 3 weeks. At some hatcheries periodic fungicide treatments are necessary throughout the holding period to prevent reinfections. Adults should be handled with PVC or rubber tubes instead of nets to reduce the incidence of fungus.

Salmon brood stock can be held in various types of holding pools including Swedish pools (oval pools), circular pools, raceways, or earthen ponds. Pools should be covered and have a freeboard of 180 cm (6 ft) or more above the water surface to prevent fish from jumping out. Water alarms and intrusion alarms should be a standard part of the holding facilities.

Water flows should be high enough to maintain oxygen levels at or near saturation and should be injected into the holding pools at an angle to create a current. A spray bar is ideal for creating a current in Swedish or circular pools. The angle of injection can be adjusted to either increase or decrease water velocity.

The number of adult salmon that can be held in a pool will depend on the holding area and available water flow but is also affected by temperature, cover, and many other factors. At the Craig Brook (Maine) National Fish Hatchery, as many as 230 fish weighing 5 kg (10 lb) each have been successfully held in a 120 m^2 (1,296 ft^2) Swedish pool, with a water depth of 60 cm (2 ft), maximum water temperature of 17°C (63°F) during the warmest month, and water flow of 400 L/min (110 gal/min). The relatively low water inflow rate was acceptable because Atlantic salmon do not feed after they enter fresh water.

Atlantic salmon are usually held 3–6 months before they mature sexually. During holding, most salmon culturists believe that brood fish should be kept in water temperatures averaging 13–16°C (55–60°F). Fluctuations ranging from 10 to 21°C (50 to 70°F) are tolerable and not uncommon.

Water for salmon brood stock should be taken from a disease-free source, such as a well or protected spring; otherwise, the water supply should be filtered and treated with ultraviolet light. Even if the water supply is disease-free, wild salmon often carry parasites and pathogenic bacteria that can cause serious losses. Culturists must be alert for any unusual behavior exhibited by the fish that may indicate a disease problem. Increased jumping (crashing) by the fish in the brood pools, unexplained repositioning in the water column, or increased gaping are signs of impending disease. Fish should be examined immediately and treated if a pathogen is discovered. As a matter of routine, all dead brood stock should be autopsied to determine the cause of death. Minimum requirements during the autopsy are kidney and gut slants, kidney smears, parasite check of body and gills, and a general examination of the internal and external condition of the fish, during which any tears, bruises, internal bleeding, or hemorrhagic areas are noted.

Captive Brood Stock

Captive brood stock are progeny from wild, sea-run Atlantic salmon that have been reared to sexual maturity in a hatchery. Progeny from captive brood stocks are considered to be domesticated; therefore, they are not recommended as brood stock. Rather, all progeny of captive brood stock are stocked as fry, parr, or smolts.

Fish are chosen for a captive brood stock by random selection from a large group of pre-smolts (see Chapter 1). The selected fish may be transferred directly into brood pools or remain in rearing pools through late spring and summer before being moved in fall. Brood pools are raceways, circular pools, Swedish pools, earthen ponds, or square pools that are covered or indoors to reduce light intensity. Pool sides must extend 180 cm (6 ft) or more above the water surface to prevent fish from jumping out. Eggs taken from fish held at densities reaching 35 kg of fish per cubic meter (2.24 lb/ft^2) had no significant reduction in hatchability compared to eggs taken from fish held at lower densities.

Captive brood stocks should be held in high quality water. At the Green Lake (Maine) National Fish Hatchery (Fig. 5.1), brood stocks produce high quality eggs at temperatures of 0.5–3°C (33–38°F) in winter and 18–19°C (65–66°F) in summer. Water temperatures vary with natural fluctuations in the lake water supply, and water flows are set so that oxygen levels remain at or near saturation. Water depth in rearing tanks is usually 90 cm (3 ft) but may vary from 45 cm (1.5 ft) to 180 cm (6 ft).

Artificial salmon diets are readily taken by captive brood stock. The open-formula ASD2-30 diet has been especially effective, providing excellent growth, feed conversion, and egg quality. Brood fish are fed to satiation, using automatic feeders, demand feeders, or hand feeding. Feeding activity is reduced as water temperatures decline, but fish continue to feed even during winter.

Captive brood stocks usually reach sexual maturity at 4 years, although a few may produce gametes as 3 year olds. The quality of eggs from first-time spawners is considerably better than that of eggs from fish spawning for a second or third time. For this reason, it is recommended that only first-time spawners be used as brood stock. A brood stock operation in which only first-time spawners are used provides higher quality eggs and savings in space, labor, and fish food.

Kelts

After sea-run salmon spawn, they are called kelts. Kelts can be held in fresh water and rejuvenated for repeat spawning after 1 or 2 years. At the Berkshire (Massachusetts) Trout Hatchery, kelts are vaccinated for enteric redmouth and furunculosis on receipt and placed in small fiberglass tanks containing about 60 cm (2 ft) of water. Water temperatures from 3 to 7°C (38–44°F) are adequate for kelt rejuvenation at the Berkshire facility, but my experience in raising salmon indicates higher temperatures may be better. Water flows are set to maintain oxygen near saturation levels.

Resumption of feeding is the most critical hurdle to overcome in kelt rejuvenation. Kelts are held undisturbed for a week after they arrive at the station before initial feeding is attempted. A food ball attached to the end of a stiff wire is placed directly in front of the fish to be fed. The food ball is made from a combination of ingredients, including herring and shrimp paste, liver, ASD2-30 starter, and vitamin and mineral supplements. Some fish take the food immediately, others require periods of coaxing. After fish take the first food, they are

Fig. 5.1. Circular pools at the Green Lake National Fish Hatchery used for rearing Atlantic salmon smolts.

enticed to take food dropped in front of them, and then food thrown directly into the holding pool. Within 1 month, most fish are feeding aggressively and can be fed to satiation. When fish are feeding normally, they are moved to circular pools, 6.7 m (22 ft) in diameter, where feeding continues until the spawning season. About 25 fish are held in each pool.

Fungicides and 1.5% salt solutions should be used on an as-needed basis to control fungus infection. Kelts remaining on-station more than 1 year should receive an annual injection of enteric redmouth and furunculosis bacterin. Egg quality declines with kelt age, in a pattern similar to that in captive stock.

Spawning Protocol

Salmonid spawning operations vary around the world. Although many of the methods produce satisfactory results, individual fish culturists are usually convinced that their methods are best and are reluctant to make changes. However, spawning practices should be standardized to permit comparison of results between hatcheries, minimize genetic inbreeding, and produce higher and more consistent egg quality. The following protocol is a step-by-step outline of successful practices used in Atlantic salmon spawning at the Craig Brook National Fish Hatchery. This protocol should yield excellent results at any spawning location, given the absence of serious problems such as disease, poor nutrition, or unsatisfactory water quality.

An important first step in any brood stock program is to maintain stock integrity. If wild salmon are used for brood stock, early- and late-run fish—as well as fish from different river systems—should be reared separately. In both wild and captive brood stock programs, fish of different age classes should also be held separately. Holding and spawning each group separately will prevent genetic contamination of unique populations and thereby help maintain the genetic diversity of the stocks.

The spawning operation begins with sectioning the spawning facility or holding pool into at least five compartments to segregate fish into five groups: males, ripe females, unripe females, barren fish, and spent fish. The sexing and sorting operations should minimize fish handling. Determine sex, and separate sexes 10–14 days before the anticipated date of ripening of the earliest maturing fish. Maturation is regulated by day length and water temperature, unless hormones are used. Check females for ripeness at 5- to 7-day intervals, or more often if the fish culturist believes the females are close to spawning. If the fish are feeding (captive brood stock), males and females should be taken off feed 4–5 days before first spawning and not be fed again until after spawning.

Eggs are taken from ripe females and fertilized using the following procedures:

1. The "dry method" of spawning (absence of water) is the recommended procedure for Atlantic salmon. The

spawning pan should be a dark color, smooth, and made of plastic, porcelain, or painted metal. The dark color aids spawn takers to see broken or inferior eggs. Black asphaltum varnish can be used, but the pans should not be made of, or coated with, heavy metals, such as zinc (galvanized). When a problem with the eggs is detected, the stream of undesirable eggs should be diverted into a separate pan and discarded. If air and water temperatures differ by more than a few degrees, steps should be taken to avoid temperature shock. One way to avoid temperature shock is to float the dry pan in a second pan containing water from the spawning pool. The "dry pan" is rinsed in clean water immediately before use and drained until water ceases to drip from the pan interior.

2. At the beginning of the spawning season, milt from several males should be examined microscopically for sperm viability. The milt is prepared by mixing with saline or ovarian fluid on a microscope slide and immediately checking for sperm motility and abnormal structure. Samples should be rechecked if poor viability is suspected. Current practice is to use milt from one male to fertilize one female. Milt from another male or pooled sperm may be added for insurance. For genetic considerations, only single-pair matings should be made when progeny will be kept for brood stock (see Chapter 2). The number of males and females used in each spawning set should always be equal to provide a 1:1 sex ratio.

Milt can be taken the day before the eggs are collected if it is properly stored and refrigerated to maintain sperm viability. One successful method of storing or transporting sperm (as suggested by Ray Simon, National Fish Health Research Laboratory) is to place about 3 mL of milt in small, thin, plastic bags (sandwich or freezer bags), seal the top, and place the bags on ice in a container filled with oxygen. The oxygen diffuses through the plastic bags and sustains sperm life. At Craig Brook National Fish Hatchery, milt collected in test tubes and held on ice remained viable for at least 12 h.

Males are either anesthetized or put in a flopping net and allowed to tire before milt is taken. During spawning, males are held in much the same manner as females; the thumb and fingers apply pressure on the sides of the fish, and the vent is pointed away from the milt collection pan. Pressure is applied by moving the hand downward toward the vent to strip the fish of urine and seminal fluid. After all urine and seminal fluids are cleared, milt is directed into the collection pan (Fig. 5.2). Care must be taken to prevent water contamination of the milt because water activates the sperm and renders it useless. Because milt is usually extruded as a stream, it is not necessary to hold the fish directly over the collection container.

3. Net four or five females, preferably one fish at a time if the fish are large, and place them into a tub of water containing 100–150 ppm of the anesthetic tricaine methanesulfonate (MS-222). Assign one member of the spawning team to add females to the anesthetic container so that the spawning operation is not delayed, and to monitor exposure time to avoid overexposure and death.

Fig. 5.2. Spawning a male Atlantic salmon.

4. After the fish are anesthetized, record the desired information: length, weight, and tag number or marks. The anesthetized fish, as well as gloves or hands that come into contact with MS-222, are rinsed in clean water before actual spawning to prevent anesthetic contamination of the eggs.

5. Grasp the female at the caudal peduncle with palm either up or down (up is more common) and with the other hand supporting the fish; gently apply pressure to the abdomen to begin the egg flow (Fig. 5.3). The fish is held, tail down, at about a 45° angle throughout the spawning operation. Eggs can be stripped using either of two methods. First, the spawner can start with pressure applied above the genital opening and move the hand downward toward the genital opening. This motion is then repeated while moving progressively forward on the fish. The second method is to apply gentle pressure forward on the fish below the dorsal fin and maintain that pressure as long as eggs flow in a steady stream. The hand is then moved gradually toward the genital opening, applying a uniform pressure as the flow of eggs decreases. After most of the eggs are removed, the hand is moved more rapidly, but still gently, down the abdomen from front to back to remove the remaining eggs.

Care must be taken to prevent damage to the eggs and fish. To avoid damage to eggs and internal organs, do not squeeze the sides of the abdomen together or exert heavy pressure against the backbone. Avoid running the hand all the way to the genital opening as this is a constricted area where pinching and excess pressure can result in broken eggs. Broken eggs in the spawning pan may reduce egg survival. If eggs are broken, the yolk contamination should be rinsed away with ovarian fluid or saline before sperm is added. Eggs should not be forced from a female if she is not fully ripe, holds her body rigid, or the vent is blocked. Forcible extrusion of eggs increases egg breakage, injures

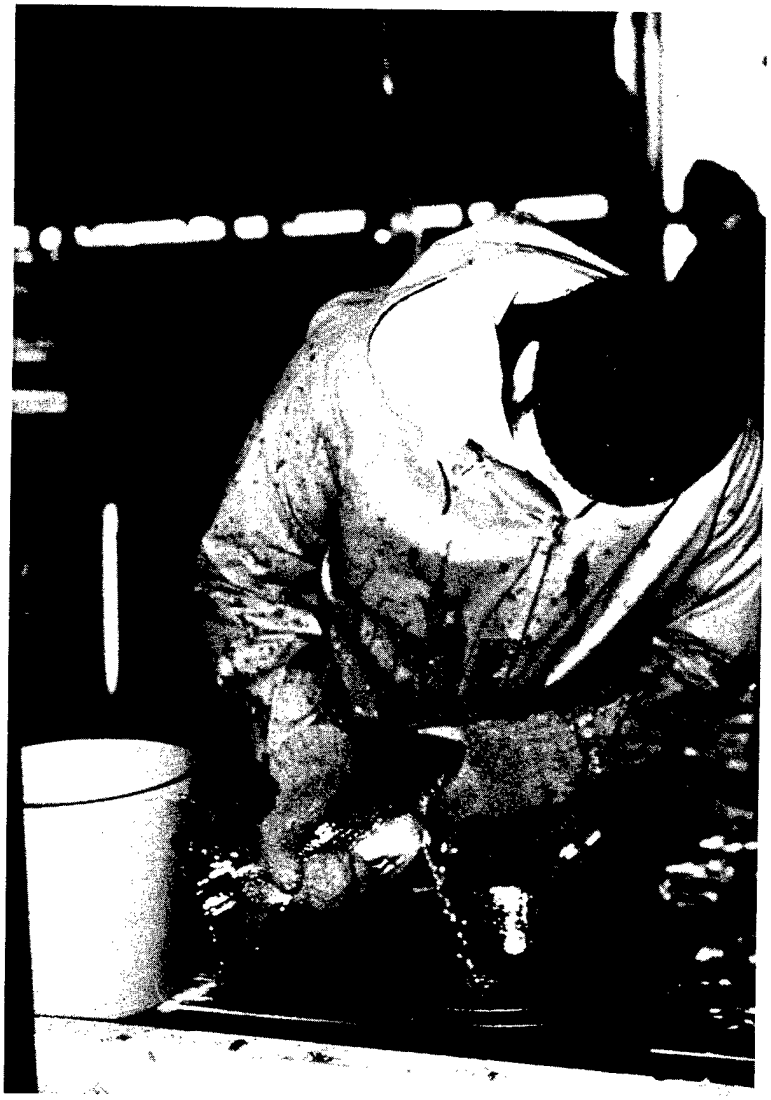

Fig. 5.3. Spawning a female Atlantic salmon.

females, and reduces fertilization. If eggs are not extruded easily, it is better to wait a few days and try again. When the vent is blocked, it may be necessary to kill the fish and open the body cavity to remove the eggs. Spent females should be placed in a separate section of the spawning pool to be rechecked in 2–3 days for additional eggs.

Air spawning is another method that may be used to extract eggs from ripe females. With this procedure, an air gun is fitted with a 0.5-in.-long needle, and air is injected directly into the body cavity of the fish, forcing eggs out the genital opening. An air compressor or cylinder, controlled by regulators set at low line pressure, provides a constant air supply to the gun. This method requires two people but is fast, clean, and less tiring than hand spawning.

I observed that the use of nets to catch eggs during extrusion, or the addition of saline, did not improve egg eye-up percentage of sea-run fish at Craig Brook National Fish Hatchery. However, these techniques have been reported to increase the percentage of eyed eggs produced by kelts and by other species.

6. During stripping every precaution should be taken to prevent water, slime, or other debris from entering the spawning pan. Hold the female so that the genital opening is over, but not touching, the edge of the pan. Be especially careful to prevent water and slime from running down the anal fin, the fish's body, or the spawn taker's glove and into the spawning pan. Grasping the female by the tail with palm down allows the spawn taker to use a forefinger to depress the anal fin away from the edge of the pan.

7. Eggs and milt can now be mixed. Milt is added to the eggs either from stored sperm or directly from the male and gently mixed by using a clean hand, soft spatula, or brush. Because sperm remains active in ovarian fluid longer than in water, it is not recommended that water be added to the egg-milt mixture. Mixing of eggs and milt should be completed immediately after the milt is added to the eggs because activated sperm survives only 15–30 s.

8. After fertilization is complete, the eggs are washed with clean water, which is then poured off to remove excess milt, debris, bad eggs, and egg shells. This flushing procedure is repeated until the eggs are clean. The clean, fertilized eggs are placed in carrying containers and fresh water is added at frequent intervals. Eggs are then transported to the egg house, where they are transferred to baskets made of netting or wire and held in flowing water for water-hardening.

9. Eggs are allowed to water-harden for about 1 h. Before transfer to incubation units, eggs are disinfected in a solution of Argentyne or Betadine (100 ppm iodine) for 10 min. If the iodophor is not already buffered, it is buffered with bicarbonate of soda until the pH of the solution approximates the pH of the water in which the eggs are held. Recent experimentation has shown that eggs can be water-hardened in the iodophor without detrimental effects. This may prove more effective in controlling some bacterial pathogens than disinfection after water-hardening. After disinfection, eggs can be allocated to incubation units. At Craig Brook National Fish Hatchery, treatment of eyed eggs with 100 ppm iodophor was limited to no more than 10 min, because longer treatment caused premature hatching and mortality. The exposure time for green eggs is less critical, even when they are treated during water-hardening.

10. Eggs should never be exposed to direct sunlight, and exposure to indirect sunlight should also be minimized. The spawning pool should be provided with a shelter to protect the eggs from direct sunlight, precipitation, and extreme temperature fluctuations. A shelter also provides a warmer and drier place for the spawn takers to work.

Recommendations

1. Brood fish should be injected with antibiotics or vaccines when furunculosis and enteric redmouth are endemic in the brood population. Antibiotics and vaccines are not recommended when brood fish are disease-free.
2. All dead brood fish should be autopsied to determine cause of death.
3. Brood stocks should be tagged so that individual fish can be identified.
4. Water temperatures should be maintained between 13 and 16°C (55 and 60°F) when possible in both captive and sea-run, brood-holding units. Oxygen levels should be maintained at or near saturation.
5. Water supply to brood-holding units should be free of pathogens.
6. Captive brood stock programs should emphasize the use of first-time spawning females.
7. Genetic integrity of each stock and year class used in the brood stock program must be maintained.
8. The dry method of egg fertilization is preferred.
9. Eggs should always be disinfected for 10 min in a 100-ppm iodine solution before incubation.

Chapter 6. Atlantic Salmon Stocking Methods

by

Victor Segarich

U.S. Fish and Wildlife Service
Nashua National Fish Hatchery
151 Broad Street
Nashua, NH 03060

Hatchery-reared Atlantic salmon are planted in river systems as fry, parr, and smolt. The life stage used depends on availability, surplus production, management objective, and habitat quality. These different stocking approaches have been examined by fishery agencies to identify the most efficient means to increase the number of adults that return from the ocean. During the initial stages of restoration, primary emphasis was placed on smolt production, and this continues to be the predominant stocking approach during the stock-building phase.

Egg Planting

Eggs can be collected, water-hardened, and then planted in stream beds or incubation boxes in or near nursery streams. Planting eggs in stream beds avoids all the problems of domestication but does not provide a significant advantage over natural spawning. Use of streamside incubators results in 74% survival through hatching but only 2% survival to emergence from the substrate in the incubator (MacKenzie and Moring 1988). Therefore, use of streamside incubators is not recommended in New England.

Each of the four stocking strategies involves intervention in the life cycle and exposure of the fish to artificial environments at several stages – egg incubation, artificial diets, exposure to fish diseases, and crowding and handling stresses. Each strategy can produce selection pressure to adapt the strain to the domestic environment. Although the logic of this argument is strong, data to demonstrate and measure the effects of rearing Atlantic salmon in the hatchery environment for one or more generations have not been published.

Fry Stocking

Fry stocking in headwater nursery areas has increased in the last few years, motivated by at least three concerns: to lessen the potential effects of domestication during hatchery life; to allow natural selection to evolve subpopulations adapted to specific upper river tributaries; and to lower costs of fish propagation and distribution.

Fry stocking normally involves transfer of fry at or before the first feeding stage into nursery areas where they are widely dispersed. This approach was the primary method employed in both the Connecticut and Merrimack river basins for Atlantic salmon restoration efforts during the nineteenth century (Rideout 1981; Stolte 1981). Although those attempts eventually failed because of inadequate fish passage facilities, adult salmon returned to the river mouth and upriver areas on the Merrimack River in substantial numbers. When the current restoration program began, fry stocking was not used extensively because barriers to migration had increased since the earlier efforts.

Advantages of fry stocking to fishery management include lower production cost per unit stocked, reduced effect of domestication on the gene pool during the hatchery production phase, and increased potential for natural selection to evolve subpopulations adapted to the separate river tributaries. Distinct subpopulations throughout the river system would evolve as variability in migratory patterns underwent natural selection.

Disadvantages of fry stocking include high natural mortality during the freshwater phase and a high frequency of 2- and 3-year smoltification. Fish mortality results from predation, adverse winter conditions, and injuries sustained during migration through power generation turbines. Smoltification is delayed due to slow growth caused by adverse water temperatures, poor water quality, and limited forage availability during parts of the year. The finding that many headwater nursery areas were capable of producing smolts from fry (Stolte 1982) was the basis for initiating a limited fry stocking program in the late 1970's. During 1983, about 40% of adult returns to the Merrimack River system were attributed to fry stockings. Fry stocking is recommended as a restoration strategy in the southern New England restoration program.

Parr Stocking

Parr – the life stage between feeding fry and smolts – are not widely stocked, because parr have most of the

disadvantages of smolts without the advantages of fry. The major disadvantage of stocking parr is that they may have adapted to the cultural environment (acclimation to artificial diets, high rearing densities, etc.) before stocking. Also, the efficiency of the natural selection cycle would be reduced by a high nonrandom mortality during adaptation from hatchery to natural freshwater environments, stress during transport, and stocking into a variable environment. They must learn to capture food almost immediately after stocking and avoid predators. Finally, large losses would be expected during migration through power facilities to the ocean. The advantages of parr stocking are protection during the fry stage and some selection for adaptation to the freshwater environment.

Smolt Stocking

Smolts – fish at or immediately before the time they are ready to migrate to sea – are widely stocked in Atlantic salmon restoration programs. The advantage is that the maximum number of fish and genetic diversity are transferred to the ocean environment for natural selection there. Adult return per fish stocked is also maximized because losses during the freshwater phase are eliminated. Disadvantages include high production costs per fish and a high potential for adaptation to the hatchery environment because of the extended time spent in the hatchery. This results in little opportunity for natural selection pressures to adapt the population to specific riverine environments (imprinting to spawning grounds, migration pattern, etc.).

Inadvertent selection during smolt production may affect the rate of change in a gene pool and alter the population in some way. The practice of producing 1-year smolts is one such example. The development rate is accelerated by elevating incubation temperature to obtain hatch 30–45 days early. Fish are then reared under standard hatchery conditions to achieve smoltification during April to May of the following year. Thus, a large percentage of fish in the population will become smolts after 1 year in the hatchery. Because the smoltification process is size dependent, hatchery production of 1-year smolts effectively selects for accelerated growth rate and ability to use artificial diets. From late March to May the fish are stocked into the river. Smolts begin the ocean migration, and slower growing parr remain in fresh water for an additional year. During that year, the parr segment of the population experiences extensive mortality from predation, fishing pressure, and overwintering that dramatically reduces the number of smolts. Also, the number of fish exposed to selection in the ocean environment is reduced relative to the 1-year smolt segment.

Smolt stocking success has been tied to fish size, age, and time of release. Investigators on both sides of the Atlantic found that fish 17–22 cm in fork length tend to produce the highest adult return rate (Carlin 1968; Ritter 1972; Peterson 1973). Hatchery-reared fish smaller than 14 cm or larger than 24 cm did not produce significant returns in tagging studies. Low survival of smaller smolts has been partly attributed to tag-related factors, whereas the poor performance of larger fish has yet to be satisfactorily explained.

Age at smolt release does not seem to affect survivability as long as fish fall within the size range of 17–22 cm (Peterson 1973; Isaksson et al. 1978). Investigations in Maine (E.T. Baum, personal communication) indicated no significant differences in return rate in age classes of similarly-sized fish, except that the larger the yearling smolt the greater the adult return; however, in 2-year smolts the return rate decreased in fish longer than 23 cm.

Timing of hatchery smolt releases has been tied to existing wild smolt migration patterns. An optimum release window has been determined for particular river systems by comparing the time of release with adult returns, but because of varying environmental and climatic factors, no clear consensus on stocking time has been reached. Current stocking practice is to release fish when water temperatures rise to 6–10°C (42–50°F) after spring runoff. Rivers in Maine have produced their highest returns when smolts are stocked during the latter part of April and the first 2 weeks in May (E.T. Baum, personal communication); more southern river systems, however, seem to produce better runs with early April stocking.

A new practice being tested by the Nashua (New Hampshire) National Fish Hatchery is the use of voluntary smolt release facilities. These elongated ponds use river water to provide natural water temperature cycles and have an escapement structure at the dam that allows the fish to determine for themselves the time to begin migration. This release system permits monitoring of optimum stocking time while reducing the effects of handling stress associated with direct release methods. Fish are also provided a longer period for stream imprinting. In Iceland, Isaksson et al. (1978) estimated that the voluntary release approach increased hatchery smolt survival 2 to 4 times over traditional truck releases.

Recommendations

1. Stock fry before the first feeding in headwater nursery habitat only if the area is devoid of wild Atlantic salmon.
2. Stock smolts as the mainstay of a restoration program during the stock rebuilding phase.
3. Plant smolts in spring when water levels and temperatures are rising.
4. Use release pools fed with river water to allow smolts to choose migration time.

Chapter 7. Atlantic Salmon Brood Stock Records

by

Thomas R. Kane

U.S. Fish and Wildlife Service
Northeast Fishery Center
P.O. Box 75
Lamar, PA 16848

Record-keeping is the basis for meaningful assessment of restoration strategies. Without records the causes of successes or failures cannot be determined. Accurate records are essential for brood stock managers if they are to incorporate genetic processes such as selection and hybridization into the management program and achieve desired results. A sound record system can also help managers avoid recurring problems in the brood stock, such as inadvertent selection or inbreeding. Records must be maintained to assess the merit of cultural practices and genetic programs and to measure progress toward achievement of restoration goals.

The degree of refinement in the brood stock record-keeping system at each station is determined by the program manager. If all fish in a brood stock are tagged, information can be maintained for each fish. This is practical in many research brood stocks or when few fish are involved. However, in production it is impossible to maintain individual data much beyond spawning. The number of fish involved and space limitations in incubation and production facilities become prohibitive when the brood stock exceeds 500 fish.

Recent advances in tagging methods – such as the Passive Integrated Transponder (PIT) and Visual Implant Tag (VIT) – have greatly reduced the incidence of lost tags and lost information compared to external tags (e.g., Carlin or Floy tags). Information from tags recovered from sea-run brood stocks can be helpful in identifying problems associated with holding facilities and handling techniques. Fecundity data collected at spawning can be correlated quickly with trapping and holding data. To the maximum extent possible, egg lots should be incubated and maintained separately by strain, spawning date, and brood stock type so that the effect of these variables can be measured in sea-run fish.

A record-keeping system should provide maximum information with minimum effort. With the availability of affordable computer equipment, it is now possible to gather and analyze a wide variety of data to quickly assess progress toward restoration goals. Efforts are under way to unify all the data collected from the New England restoration program into a single information system. Brood stock data on year class size, fish size, egg size, fecundity, disease status, spawning time, egg survival, and fry survival will be elements of the information system. Three variables – river, brood stock type, and population size – are basic if managers are to classify individual spawners and consider genetic goals in the management of brood stock sources.

River

Due to the high level of imprinting shown by salmon, the stream of origin is the primary consideration used to define the breeding population. Fishery agencies involved in Atlantic salmon restoration have placed nonindigenous stocks in formerly barren rivers. Nearby sources were available for reintroduction into Maine's Penobscot River, but initial efforts in southern New England relied on Canadian and Penobscot River stocks as the brood stock sources. As restoration is achieved and reproducing populations become established, it is anticipated that adapted strains will evolve for each of the river systems currently being stocked. Tracking the performance of non-native strains in a river system permits biologists to assess the contribution of each strain to the developing river stocks.

Brood Stock Type

Five types of brood stocks are recognized in the restoration program:

Sea-run – Maturing adults that have completed the ocean migration are trapped during their spawning run to fresh water and held in the hatchery until sexual maturity.

Kelt – Adults from previously captured sea-run brood stocks that have been reconditioned and held in the hatchery for a second or third spawning cycle.

Captive – Progeny of sea-run adults that have been reared to maturity in fresh water.

Precocious parr – Males from pre-smolt hatchery production lots that are used as a sperm source when sperm from sea-run or captive adults is not available.

Pen-reared – Adults that have been reared to maturity in saltwater sea pens.

Progeny from each of these brood stock types produced for stocking in restoration programs are routinely maintained as separate lots. The resulting lot performance information contributes to our understanding of strain differences and the effects of hatchery practices on the performance of the fish after stocking as smolts.

Population Size

Population size is the number of males and females used to produce progeny lots. The minimum number of parents contributing to each year class is important because it determines the potential for inbreeding in that brood stock. When lots are produced that include crosses of fish from different strains or brood stock types, the number of parents of each type contributing to that particular lot is recorded. The lot is then maintained separately for as long as it is physically practical before it is pooled with others into production lots.

Lot Identification

A standard protocol must be used to identify lots throughout the production cycle if brood stock information is to be integrated with restoration assessment data. Atlantic salmon lots are currently assigned names that identify the year class, parental strain, primary brood stock facility, brood stock type, and production program (Kane and Gaston 1987; Table 7.1). Such names are used for all brood stock lots, incubating egg lots, and production fish lots. For example, sea-run Penobscot River males trapped in 1988 and held at Craig Brook National Fish Hatchery – 88PCBS – might be mated with 1984-year-class domestic Union River females from Green Lake National Fish Hatchery – 84UGLD – to produce an egg lot that would be identified as 84UPGLDS. When the resultant fry begin feeding in 1989, the production lot for a 1-year smolt program would be renamed 89UPGLDS1. By using descriptive lot names, the genetic history of a lot is available every time information is reported for that lot. Subsequent information generated about the performance of a lot can then be related to the characteristics of the parent brood stock. An integrated information management system can trace lot performance data from returning adults back to the original incubation lot and the contributing parents.

Table 7.1. *List of codes used in Atlantic salmon lot designations to identify fish strain, fish rearing facility, brood stock type, and type of production program.*

Strain	Facility	Type	Program
C —Connecticut	BS —Berkshire National Fish Hatchery	D —Domestic	1 —1-year smolts
J —St. John	CB —Craig Brook National Fish Hatchery	H —Precocious parr (hatchery)	2 —2-year smolts
K —Kennebec	GL —Green Lake National Fish Hatchery	K —Kelt	f —fry stocking
M—Merrimack	NA —North Attleboro National Fish Hatchery	P —Pen reared (sea pen)	p —parr stocking
P —Penobscot	NS —Nashua National Fish Hatchery	S —Sea-run	
R —Pawcatuck	WR—White River National Fish Hatchery	X —Unknown	
U —Union	SU —Cronin National Salmon Station	Z —Mixed	
X —Unknown	TL —Tunison Laboratory		
Z —Mixed	KE —Kensington State Fish Hatchery		
	PF —People's Forest State Fish Hatchery (Whittemore)		
	FY —Perryville State Fish Hatchery		
	RR —Roger Reed State Fish Hatchery		
	MQ—Mactaquac (Canada)		
	SC —Salmon Research Centre, St. Andrews (Canada)		
	FF —New England Fish Farming Enterprises (Commercial)		
	OP —Ocean Products, Inc. (Commercial)		
	XX —Unknown		
	Z —Mixed		

Chapter 8. Atlantic Salmon Breeding Research Needs

by

Harold L. Kincaid

U.S. Fish and Wildlife Service
National Fishery Research and Development Laboratory
R.D.#4, Box 63
Wellsboro, PA 16901

The Atlantic salmon restoration program in the northeastern United States has made significant gains during the past 20 years in establishing runs to the Connecticut, Merrimack, Pawcatuck, Penobscot, and St. Croix river systems. These gains have been possible largely because of developments and increased efficiency in fish culture. These developments include the Atlantic salmon diet, kelt rejuvenation, domestic brood stock, temperature control of incubation and fry production, covered raceways, and fry stocking. Initially, fish produced for restoration stocking were 2-year smolts, but emphasis gradually turned to 1-year smolts as the technology developed. When smolt production began to reach hatchery production limits in the early 1980's, the surplus fish were planted as fry in streams uninhabited by Atlantic salmon. These technological developments based on past research have produced advances in salmon breeding that now require additional studies.

Several problems and assumptions need to be investigated. The objective of Atlantic salmon restoration is the development of natural runs through the stocking of hatchery fish and subsequent natural selection to evolve naturally-spawning brood stocks adapted to individual river systems. This is an untested hypothesis. Although this approach is theoretically sound, several generations will be needed for a natural population to evolve in a given river system from strains introduced throughout the past 20 years. The Atlantic salmon restoration program also has a number of fishery management situations that hinder progress toward restoration goals; that is, high mortality from fishing pressure in the ocean and estuary fisheries that reduce ocean returns to less than 1%, high mortality (50–95%) of smolts and pre-smolts as they pass through power-generating turbines during migration to the ocean, and difficulty for returning adults to freely migrate past dams to native spawning areas. The restoration program has attempted to overcome these obstacles by increasing the number of fish stocked, hoping this will increase the number of fish returning from the ocean. Sea-run adults are used as brood stock for succeeding production cycles, because they have undergone selection for adaptation to the natural environment during marine migration.

Examination of the probable genetic consequences of past and present management practices used in the Atlantic salmon restoration program raises a number of questions for which answers are not readily available. Current practices have been adopted because they contribute to the efficiency of hatchery or stocking operations, lower costs of production, or improve quality of the hatchery product; in most cases, information on the long-term effect of these practices on the gene pool of the developing natural population is lacking. Some hatchery practices seem to selectively favor genotypes adapted to hatchery environments, but definitive experimental data are not available to verify this conclusion. Studies are needed to further our knowledge in three general fields that affect the choice of Atlantic salmon breeding methods and cultural practices: genetic diversity, strain characterization, and evaluation of hatchery practices.

Genetic Diversity

Information is needed on genetic variability in Atlantic salmon strains used throughout the New England Atlantic salmon restoration program (Moller 1970): What are the genetic variability levels in Atlantic salmon strains being developed in the restoration fisheries? Are genetic variability levels in these strains adequate for a successful restoration program? Electrophoretic isozyme analysis has been used extensively in other fish species to measure genetic variability (heterozygosity) and should be applied to the Atlantic salmon strains. Survey studies conducted in a few Atlantic salmon strains in New England suggest that genetic variability in and between strains is very low. One comparison of the Union River strain from Maine and a wild strain from northern Sweden, using 47 loci,

showed the wild Swedish strain to have six times more heterozygosity (H. Booke, personal communication). Records from the Connecticut River program from 1977 to 1983 (Table 1.4) show that 10 different strains were stocked. At first glance, this suggests that each of these strains contributed to the genetic variability of the evolving natural strain. However, most of these strains were stocked only 1–3 years and then discontinued because of poor return rates; therefore, it is questionable if any, except the Penobscot strain, actually contributed to the genetic variability of the evolving Connecticut strain. The few fish returning to the Connecticut River between 1975 and 1984 (Table 1.1) constituted less than 100 fish in most years, and they could possibly be from only one strain.

Circumstantial evidence from isozyme studies and ocean return records suggests that genetic variability in Atlantic salmon restoration populations may be very small. To address the question of genetic variability I recommend that:

1. Atlantic salmon strains used in the New England restoration program be surveyed to obtain estimates of genetic variability in each. This survey, in which electrophoretic isozyme analysis and mitochondrial DNA techniques should be used, would provide baseline data on restoration strains and permit future monitoring of changes in genetic variability as evolution adapts natural strains.
2. A plan should be developed for the systematic introduction of new genetic variation into the Connecticut River strain by using strain crosses. Fish produced from matings of introduced strains (as the male) and Connecticut River sea-run (as the female) could be stocked as a designated fraction of scheduled plants. This would achieve the goal of gradual expansion of the gene pool at rates high enough to increase genetic variability but low enough to avoid overpowering the evolving populations.

Strain Characterization

A method to permit positive identification of individual strains is urgently needed. Currently, there is little evidence to indicate that managed brood stocks are different strains. Nevertheless, fish from each river system (Connecticut, Merrimack, and Penobscot) and brood stock type (domestic, sea-run, and kelt) are held and managed separately on the assumption that they are genetically different groups. A measurable basis (physical appearance, hatchery or field performance, isozyme pattern, etc.) is needed to characterize each strain. I recommend that:

1. Studies be designed to characterize strains, to establish criteria for defining strains, and to determine those Atlantic salmon populations that constitute unique genetic strains.
2. A system of marking (e.g., coded wire tags) be developed to evaluate field performance of different putative strains.

Evaluation of Hatchery Practices

The effect of hatchery production practices on the performance of fish after release needs to be evaluated in terms of fish survival, growth rate, and reproductive capacity, using an approach similiar to that of Hosmer et al. (1979). Stocking practices also affect adult returns; smolts planted during high water returned at a greater rate than those planted during low water (Hvidsten and Hansen 1988). Many of the current cultural and management practices used in Atlantic salmon production have not been evaluated. Production of 1-year smolts is just one example of a hatchery practice expected to have long-term effects on the gene pool. Production of 1-year smolts is, in effect, selecting against the slower-growing fish and leads to a reduction in genetic variability. Various field studies should be conducted to measure the effect of hatchery practices on post-stocking performance, such as:

1. The survival of 1-year smolts, exposed to an accelerated growth environment, relative to the 2-year smolt.
2. The growth and survival of progeny produced from domestic brood stock relative to progeny from sea-run and kelt brood stocks in hatchery and field environments.
3. The total return rate (to the river) after ocean migration of fish stocked as fry, parr, and smolts.
4. The relation between the length of time fish are cultured in the hatchery and changes in genetic variability.
5. The relation between rearing density in the hatchery and ocean return rate.

Goal of Atlantic Salmon Breeding

Atlantic salmon restoration in New England rivers has come a long way in only 20 years; however, much more work will be needed before the restoration effort is completed. Populations returning from the ocean have been established in at least five river systems. The challenge now, in each of these rivers, is to increase the annual returns from the current few hundred to several thousand. Current sea-run populations rely on hatchery production, which will continue to be the primary method for enhancement of Atlantic salmon until self-sustaining populations evolve. Hatchery fish can perform in natural environments if hatchery cultural practices do not select against traits favorable to survival, growth, and reproduction in the natural environment. Expansion of newly established Atlantic salmon runs will depend on the ability of managers to provide quality fish that are healthy and possess the

genetic diversity to adapt to river, estuary, and ocean environments. Sound breeding practices that maintain high levels of genetic variability and minimize selection in the hatchery environment will help protect the existing Atlantic salmon gene pools as they evolve into established naturally spawning strains.

Recommendations

1. Accelerate the production of 1-year smolts by using heated water and improving culture techniques so that all fish smoltify after 1 year. Such a program would minimize selection for genetic traits governing growth.
2. Experiment with saturation stocking to promote faster restoration; stock barren streams with fry; then, at the time of migration, add smolts. This would increase the number of returning adults to a critical population size.
3. Provide overhead cover for fry-rearing tanks to increase growth rate by decreasing stress from human activities (Pickering et al. 1987).

References

Allen, S. K., Jr., and J. G. Stanley. 1979. Polyploid mosaics induced by cytochalasin B in landlocked Atlantic salmon, *Salmo salar*. Trans. Am. Fish. Soc. 108:462–466.

Allen, S. K., Jr., and J. G. Stanley. 1981. Polyploidy and gynogenesis in the culture of fish and shellfish. Int. Counc. Explor. Sea C.M. 1981/F:28. 18 pp.

Arai, K., and N. P. Wilkins. 1987. Triploidization of brown trout (*Salmo trutta*) by heat shocks. Aquaculture 64:97–103.

Bardach, J. E., J. H. Ryther, and W. O. McLarney. 1972. Aquaculture, the farming and husbandry of freshwater and marine organisms. Wiley-Interscience, New York. 870 pp.

Beland, K. F., F. L. Roberts, and R. L. Saunders. 1981. Evidence of *Salmo salar* × *Salmo trutta* hybridization in North American rivers. Can. J. Fish. Aquat. Sci. 38:552–554.

Carlin, B. 1968. Salmon tagging experiments. Atlantic Salmon Association Centennial Award Fund Special Publication. University of New Brunswick, Fredericton, N.B. February 1968. 22 pp.

Carlson, A. R., and J. C. Hale. 1973. Early maturation of brook trout in the laboratory. Prog. Fish-Cult. 35:150–153.

Chadwick, E. M. P. 1985. Fundamental research problems in the management of Atlantic salmon, *Salmo salar* L., in Atlantic Canada. J. Fish Biol. 27A:9–25.

Cherfas, N. B. 1975. Investigations of radiation-induced diploid gynogenes in the carp (*Cyprinus carpio* L.). I. Experiments on obtaining the diploid gynogenetic progeny in mass quantities. Genetika 11:78–86. (in Russian)

Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (*Salmo gairdneri* Richardson). Reprod. Nutr. Dev. 20:727–733.

Crim, L. W., B. D. Glebe, and A. P. Scott. 1986. The influence of LHRH analog on oocyte development and spawning in female Atlantic salmon, *Salmo salar*. Aquaculture 56:139–149.

Donaldson, E. M., J. D. Funk, F. C. Withler, and R. B. Morley. 1972. Fertilization of pink salmon (*Oncorhynchus gorbuscha*) ova by spermatozoa from gonadotropin-injected juveniles. J. Fish. Res. Board Can. 29:13–18.

Donaldson, E. M., and G. A. Hunter. 1982. Sex control in fish with particular reference to the salmonids. Can. J. Fish. Aquat. Sci. 39:99–110.

Doyle, R. W. 1983. An approach to the quantitative analysis of domestication selection in aquaculture. Aquaculture 33:167–185.

Falconer, D. S. 1981. Introduction to quantitative genetics. 2nd ed. Longman Inc., New York. 342 pp.

Graham, M. S., G. L. Fletcher, and T. J. Benfey. 1985. Effect of triploidy on blood oxygen content of Atlantic salmon. Aquaculture 50:133–139.

Hershberger, W. K., K. Bonham, and L. R. Donaldson. 1978. Chronic exposure of chinook salmon eggs and alevins to gamma irradiation: effects on their return to freshwater as adults. Trans. Am. Fish. Soc. 107:622–631.

Hosmer, M. J., J. G. Stanley, and R. W. Hatch. 1979. Effects of hatchery procedures on later returns of Atlantic salmon to rivers in Maine. Prog. Fish-Cult. 41:115–119.

Hvidsten, N. A., and L. P. Hansen. 1988. Increased recapture rate of adult Atlantic salmon, *Salmo salar* L., stocked as smolts at high water discharge. J. Fish Biol. 32:153–154.

Isaksson, A., T. Rasch, and P. H. Poe. 1978. An evaluation of smolt releases into a salmon and non-salmon producing stream using two release methods. J. Agric. Res. Iceland 10:100–113.

Jensen, A. J., and B. O. Johnsen. 1986. Different adaptation strategies of Atlantic salmon (*Salmo salar*) populations to extreme climates with special reference to some cold Norwegian rivers. Can. J. Fish. Aquat. Sci. 43:980–984.

Johnstone, R., T. H. Simpson, and A. F. Youngson. 1978. Sex reversal in salmonid culture. Aquaculture 13:115–134.

Kane, T. R., and P. B. Gaston. 1987. Lot naming guidelines for Atlantic salmon. U.S. Fish Wildl. Serv., Lamar Inf. Leafl. #88-03. 9 pp.

Kincaid, H. L. 1976. Effects of inbreeding on rainbow trout populations. Trans. Am. Fish. Soc. 105(2):273–280.

Kunesh, W. H., W. J. Freshman, M. Hoehm, and N. G. Nordin. 1974. Altering the spawning cycle of rainbow trout by control of artificial light. Prog. Fish-Cult. 36:225–226.

Lincoln, R. F., D. Aulstad, and A. Grammeltvedt. 1974. Attempted triploid induction in Atlantic salmon (*Salmo salar*) using cold shocks. Aquaculture 4:287–297.

Lincoln, R. F., and A. P. Scott. 1983. Production of all-female triploid rainbow trout. Aquaculture 30:375–380.

MacCrimmon, H. R., and R. R. Claytor. 1985. Meristic and morphometric identity of Baltic stocks of Atlantic salmon (*Salmo salar*). Can. J. Zool. 63:2032–2037.

MacKenzie, C., and J. R. Moring. 1988. Estimating survival of Atlantic salmon during the intragravel period. N. Am. J. Fish. Manage. 8:45–49.

MacKinnon, C. N., and E. M. Donaldson. 1976. Environmentally induced precocious sexual development in male pink salmon (*Oncorhynchus gorbuscha*). J. Fish. Res. Board Can. 33:2602–2605.

McEvoy, T., M. Stack, B. Keane, T. Barry, J. Sreenan, and F. Gannon. 1988. The expression of a foreign gene in salmon embryos. Aquaculture 68:27–37.

Moller, D. 1970. Genetic diversity in Atlantic salmon and salmon management in relation to genetic factors. Int. Atl. Salmon Found. Spec. Publ. Ser. 1:5–29.

Naevdal, G. 1981. Fish rearing in Norway with special reference to genetic problems. Pages 85–93 *in* N. Ryman, ed. Fish gene pools. Ecol. Bull. (Stockholm) 34.

Oppermann, K. 1913. Die Entwicklung van Forelleneiern nach Befruchtung mit radiumbestrahlten Samenfaden. Arch. Mikroskop. Anat. 83:181–189.

Peterson, H. H. 1973. Adult returns to date from hatchery-reared one-year-old smolts. Pages 219–226 *in* M. W. Smith and W. M. Carter, eds. Int. Atl. Salmon Found. Spec. Publ. Ser. 2.

Pickering, A. D., R. Griffiths, and T. G. Pottinger. 1987. A comparison of the effects of overhead cover on the growth, survival and haematology of juvenile Atlantic salmon, *Salmo salar* L., brown trout, *Salmo trutta* L., and rainbow trout, *Salmo gairdneri* Richardson. Aquaculture 66:109–124.

Pirchner, F. 1983. Population genetics in animal breeding. Plenum Press, New York. 408 pp.

Purdom, C. E., and R. F. Lincoln. 1973. Chromosome manipulation in fish. Pages 83–89 *in* J. H. Schroder, ed. Genetics and mutagenesis of fish. Springer-Verlag, Berlin.

Refstie, T., V. Vassvik, and T. Gjedrem. 1977. Induction of polyploidy in salmonids by cytochalasin B. Aquaculture 10:65–74.

Reisenbichler, R. R. 1988. Relation between distance transferred from natal stream and recovery rate for hatchery coho salmon. N. Am. J. Fish. Manage. 8:172–174.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations. Pages 19–160 *in*

R. C. Simon and P. A. Larkin, eds. The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries. University of British Columbia, Vancouver.

Rideout, S. G. 1981. Restoration of Atlantic salmon to the Connecticut River basin 1967–1981. Int. Counc. Explor. Sea C.M. 1981/F:29. 10 pp.

Riddell, B. E., W. C. Leggett, and R. L. Saunders. 1981. Evidence of adaptive polygenic variation between two populations of Atlantic salmon (*Salmo salar*) native to tributaries of the S.W. Miramichi River, N.B. Can. J. Fish. Aquat. Sci. 38:321–333.

Ritter, J. A. 1972. Preliminary observations on the influence of smolt size on tag return rate and age at first maturity of Atlantic salmon (*Salmo salar*). Int. Counc. Explor. Sea. C.M. 1972/M:14. 10 pp.

Ritter, J. A. 1975. Lower ocean survival rates for hatchery-reared Atlantic salmon (*Salmo salar*) stocks released in rivers other than their native streams. Int. Counc. Explor. Sea C.M. 1975/M:26. 10 pp.

Saunders, R. L. 1981. Atlantic salmon (*Salmo salar*) stocks and management implications in the Canadian Atlantic Provinces and New England, USA. Can. J. Fish. Aquat. Sci. 38:1612–1625.

Schreck, C. B. 1974. Hormonal treatment and sex manipulation in fishes. Pages 84–106 *in* C. B. Schreck, ed. Control of sex in fishes. Virginia Polytechnical Institute and State University, Blacksburg, Va.

Secombes, C. J., L. M. Laird, and I. G. Priede. 1987. Immunological approaches to control maturation in fish. II. A review of the autoimmune approach. Aquaculture 60:287–302.

Shields, W. M. 1982. Philopatry, inbreeding and the evolution of sex. State University of New York Press, Albany, NY.

Sower, S. A., C. B. Schreck, and E. M. Donaldson. 1982. Hormone-induced ovulation of coho salmon (*Onchorhynchus kisutch*) held in seawater and freshwater. Can. J. Fish. Aquat. Sci. 39:627–632.

Stanley, J. G. 1981. Manipulation of developmental events to produce monosex and sterile fish. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 178:485–491.

Stolte, L. W. 1981. The forgotten salmon of the Merrimack. U.S. Fish and Wildlife Service, Boston, Mass. 214 pp.

Stolte, L. W. 1982. A strategic plan for restoration of Atlantic salmon to the Connecticut River Basin. U.S. Fish and Wildlife Service, Boston, Mass. 81 pp.

Stoss, J., S. Buyukhatipoglu, and W. Holtz. 1978. Short-term and cryopreservation of rainbow trout (*Salmo gairdneri* Richardson) sperm. Ann. Biol. Anim. Biochim. Biophys. 18:1077–1082.

Svardson, G. 1945. Chromosome studies on salmonidae. Medd. Unders. ForstAnst. SottvattFisk. 23:1–151.

Thorgaard, G. H., M. E. Jazwin, and A. R. Stier. 1981. Polyploidy induced by heat shock in the rainbow trout. Trans. Am. Fish. Soc. 110:546–550.

Wright, S. 1922. Coefficient of inbreeding and relationship. Am. Nat. 56:330–338.

Yamamoto, T. 1969. Sex differentiation. Pages 117–175 *in* W. S. Hoar and D. J. Randall, eds. Fish physiology. Vol. 3. Academic Press, New York. 485 pp.

Zell, S. R. 1978. Cryopreservation of gametes and embryos of salmonid fishes. Ann. Biol. Anim. Biochim. Biophys. 18:1089–1099.

Appendix. Glossary

Alevin (sac fry) Young salmon from hatch through absorption of yolk sac to independent feeding.

Adaptation The process by which organisms or populations of organisms adjust to function more effectively in a given environment.

Additive effect (additive genetic effect) Average effect of substituting one allele for its alternative allele in a given population.

Additive variance Genetic variance due to the additive effects of genes.

Albinism The condition resulting from the inability of an animal to form pigment in the eyes, skin, and other tissues; generally inherited as an autosomal recessive.

Alleles Pairs of a gene situated at the same locus in homologous chromosomes that express alternative characters. When more than two forms of a gene exist, the locus is said to show multiple allelism.

Artificial selection The choosing by humans of individual fish with specific genotypes that will contribute to the gene pool of succeeding generations.

Assortative mating Sexual reproduction in which the pairing of male and female is not random, but involves a tendency for males of a particular kind to breed with females of a particular kind. Positive (or negative) assortative mating occurs if parents tend to be more (or less) alike than is expected by chance alone.

Autosome A chromosome that is not a sex chromosome.

Backcross The cross of an F_1 heterozygote with one of the two parents or with an individual of the same genotype as a parent.

Backcross breeding A system of breeding where recurrent backcrosses are made to one of the parents of a cross, accompanied by selection for a specific character or characters.

Breeding value The genetic value of an individual measured as the mean performance of its progeny for a specific trait or traits.

Bright salmon A fish that enters fresh water to start a spawning migration.

Brood stock A fish population living at a single location (hatchery, stream, lake) as a self-sustaining interbreeding unit, maintained either naturally or artificially.

Captive brood stock A brood stock held in a hatchery environment throughout its life but whose parents were reared to adulthood in the natural environment.

Cell The smallest membrane-bound protoplasmic unit capable of independent reproduction.

Character An attribute of an organism resulting from the interaction of a gene or genes with the environment.

Chromosome A linear structure of cell nuclei containing DNA, responsible for the determination and transmission of hereditary characteristics.

Clone A group of genetically identical individuals descended from a single common ancestral cell by mitosis.

Codominant Condition whereby both alleles in a heterozygote express their products phenotypically.

Combining ability *1. General combining ability*: Average performance of an individual, line, or strain in a series of crosses. *2. Specific combining ability*: Deviation in performance of a particular cross from that predicted on the basis of general combining ability.

Consanguinity Relationship between individuals that are descendants of a common ancestor. Consanguineous matings are matings between relatives.

Cull The identification and discarding of inferior animals (individuals) from a breeding stock.

Cytoplastic inheritance Transmission of hereditary characters through the cytoplasm, as compared with transmission by genes carried on chromosomes; detected by generally greater or exclusive contribution of female phenotype to the progeny in reciprocal crosses.

Dam The female parent in animal breeding.

Deme A local interbreeding group of organisms.

Diallel cross The crossing in all possible combinations of a series of genotypes (individuals, lines, or strains).

Dihybrid A cross between individuals that differ with respect to two specific gene pairs.

Diploid A cell, tissue, or organism with two complete sets of chromosomes ($2n$).

Directional selection A selection process acting on a population that results in a shift in the mean of a trait in the direction desired by the breeder.

Domestic brood stock A brood stock maintained by humans in a hatchery environment (tanks, raceways, or ponds) for at least two generations.

Dominance Condition where one allele partly or completely masks the expression of its alternative allele when they are in the heterozygous state. An allele shows complete dominance if its phenotypic effect is the same in both the heterozygous and homozygous states. An allele expresses incomplete or partial dominance if the heterozygous phenotype is intermediate between the homozygous dominant and a hypothetical phenotype midway between the homozygous states.

Donor parent The parental source of one or more genes to be transferred to the progeny in a backcross breeding program.

Double-cross hybrid A cross between two F_1 (single-cross) hybrids.

Drift See **Genetic drift (random genetic drift)**.

Effective population size The number of individuals in a population that would yield the same calculated sampling variance or rate of inbreeding as that found in an ideal random mating population whereby every individual has an equal probability of contributing to the next generation. Effective population size is calculated by the equation:

$$N_e = (4N_mN_f) \div (N_m + N_f)$$

where N_e = effective population size; N_m = actual number of males; and N_f = actual number of females.

Electrophoresis A technique that separates charged molecules in a solution in an electrical field. The solution is generally held in a porous supporting medium such as filter paper, cellulose acetate, or a gel of starch, agar, or polyacrylamide.

Environment The sum total of all external conditions and influences that affect behavior, growth, and development of an organism.

Epistasis All types of genetic interactions involving alleles at two or more different loci.

Evolution The cumulative change in the characteristics of populations of organisms related by descent that occur during the course of successive generations over a long time.

Expressivity The degree to which a gene produces a phenotypic effect.

F_1 The first filial generation. The first generation of descent from a given parental mating.

F_2 The second filial generation obtained by crossing two individuals of the first filial (F_1) generation. It is two generations distant from the parental mating.

F_3 The third filial generation obtained by crossing two individuals of the second filial (F_2) generation. It is three generations distant from the parental mating.

Family A group of individuals directly related by descent from a common ancestor. Scope of the family is determined by the common ancestor. (see **Siblings**)

Fecundity In egg-producing species, the term refers specifically to the number of eggs produced by a female over a defined time.

Fertility Ability to produce viable offspring.

Fertilization Fusion of the nuclei of male and female gametes to produce a zygote.

Fitness (Darwinian fitness) The relative ability of an organism to survive and transmit its genes to the next generation.

Founder effect Genetic drift caused by starting a population with a small number of individuals.

Fry Transitional stage in natural development of fish from emergence from egg to dispersal from the area of the redd.

Gamete Cell of meiotic origin specialized for fertilization. The male or female reproductive cell (sperm or egg).

Gametic number The number of chromosomes contained in the gamete. Normally this is the haploid number of chromosomes, symbolized by n.

Gametogenesis The process by which gametes are formed.

Gene The hereditary unit that transmits a genetic specification from one generation to the next; a segment of DNA coding for one function or several related functions. A gene can mutate to various allelic forms.

Gene frequency The proportion, usually expressed as a decimal fraction, in which alternative alleles of a gene occur in a population.

Gene interaction Modification of a gene's expression by the influence of a nonallelic gene or genes (also called epistasis).

Gene pool Total of all possible genes (alleles) in the reproductive gametes of a population.

Generation interval Time between corresponding stages of the life cycle in successive generations (average age of reproducing adults).

Genetic drift (random genetic drift) The random fluctuation of gene frequencies from generation to generation due to chance fluctuation. Although drift occurs in all populations, its effects are most evident in small populations.

Genetic equilibrium The condition in which successive generations of a population contain the same genotypes in the same proportions with respect to individual genes and gene combinations.

Genetic homeostasis The tendency of a population to maintain genetic variability despite forces such as selection and inbreeding that act to reduce variability.

Genetic variance The portion of the phenotypic variance caused by the different genotypes of the individuals in a population.

Genetics The field of biology that deals with heredity and its variation.

Genome A complete set of chromosomes corresponding to the haploid (n) set of a species.

Genotype The genetic constitution of an individual, as distinguished from its physical appearance (phenotype). May be used to refer to either a single locus or the entire individual.

Genotype–environment interaction The differential response of a genotype in different environments.

Grilse A sexually maturing salmon that returns to its natal river after one winter in the sea.

Hanks' balanced salt solution A solution of salts that matches the concentrations of body fluids; commonly used in tissue culture.

Haploid One complete set of chromosomes, symbolized by n. Corresponds to a genome or the gametic chromosome number in diploid species.

Hardy-Weinberg Law The law stating that gene frequencies and genotype frequencies remain constant from generation to generation in large populations that mate at random, provided there is no selection, migration, or mutation. In the situation where a single pair of alleles (A and a) is considered, the frequencies of gametes carrying A and a are defined as p and q, respectively, and the genotypic equilibrium frequencies are p^2 (AA), $2pq$ (Aa), and q^2 (aa).

Heredity The sum of qualities and characteristics transmitted from parent to progeny through the gametes.

Heritability (h^2) A measure of the degree to which a phenotype is genetically influenced and can be modified by selection. It is defined by the equation $h^2 = \sigma^2_a \div \sigma^2_p$, where σ^2_a is the variance due to genes with additive effects and σ^2_p is the phenotypic variance.

Hermaphrodite An individual capable of producing both male and female gametes.

Heterogametic The sex whose gametes differ in the kinds of sex chromosomes that can be transmitted.

Heterosis (hybrid vigor) The increased vigor in quantitative traits (e.g., growth, survival, fertility) of hybrids over both of the parents in crosses between strains, brood stocks, or inbred lines. Heterosis is associated with increased heterozygosity.

Heterozygosity The condition of having unlike alleles at one or more loci.

Heterozygote Individual having unlike alleles at one or more corresponding loci; opposite of homozygote.

Homogametic The sex whose gametes do not differ in the kind of sex chromosomes that can be transmitted to progeny.

Homologous chromosomes Chromosomes that occur in pairs and are identical with respect to constituent loci and visible structure.

Homozygosity The condition of having identical alleles at one or more loci.

Homozygote Individual that possesses identical alleles at corresponding loci of a chromosome pair.

Hybrid The progeny of a cross between genetically unlike parents; normally applied to crosses between species or inbred lines.

Hybrid vigor Same as heterosis.

Inbred line A line produced by continued mating of relatives (inbreeding) over a number of generations, normally by selfing, brother-sister mating, mother-son mating, or father-daughter mating.

Inbreeding The mating of individuals that are more closely related to each other than are randomly mating individuals within a population.

Inbreeding coefficient (F) *1.* Probability that two alleles at one locus in a zygote are derived from a common ancestor. *2.* Expected percent decrease in heterozygosity, relative to a specific base population, that has been caused by the mating of relatives.

Inbreeding depression Reduction in fitness or vigor due to inbreeding.

Independent assortment The random distribution to the gametes of genes on different chromosomes. Thus, an individual of genotype $AaBb$ is expected to produce equal numbers of four types of gametes: AB, Ab, aB, and ab. (see **Mendel's Laws**)

Intersex Individual from a normally bisexual species with reproductive organs or secondary sex characteristics that are partly of one sex and partly of the other.

Isolation The separation of one group from another that prevents the mating of individuals from the two groups.

Kelt A spent or spawned-out salmon found in the freshwater portion of a river system.

Line (inbred line) A population developed from the mating of close relatives, especially brother and sister, half-brother and half-sister, mother and son, or father and daughter.

Linkage An association in inheritance of two or more nonallelic genes that is greater than expected from independent assortment. Genes are linked because they are on the same chromosome.

Locus The physical position occupied by a gene on the chromosome.

Marker An allele whose phenotype is observed in crosses.

Mass selection A selection procedure in which individuals or families that are superior for one or more traits are selected from the general population for use as parents to the next generation. (see **Truncation selection**)

Maternal effects Phenotypic differences between individuals that are caused by the effect of the female parent in producing the egg and carrying the developing zygote. (see **Cytoplasmic inheritance**)

Mating system Any scheme by which individuals are assorted in pairs, leading to sexual reproduction. *1. Random mating*—assortment of individuals into mating pairs by chance. *2. Genetic assortative mating*—mating of individuals that are more closely related than are randomly chosen individuals from the general population. *3. Genetic disassortative mating*—mating of individuals that are less closely

related than are randomly chosen individuals from the general population. *4. Phenotypic assortative mating*—mating individuals that are more alike in appearance than the average of the population. *5. Phenotypic disassortative mating*—mating of individuals that are less alike in appearance than the average of the population.

Meiosis The cellular reduction division process by which diploid cells produce haploid cells during gamete formation.

Mendel's Laws Fundamental laws governing the inheritance of single locus traits: *1. The law of segregation*. Alleles occur in pairs in the cells of individuals. When gametes are produced, the members of a pair separate so that each gamete receives only one member of the pair. *2. The law of independent assortment*. Transmission of alleles at one locus to the gametes does not influence the transmission of alleles at other loci into the gametes during gametogenesis. This law applies only to genes on different chromosomes. (see **Linkage**)

Migration Mechanism for introducing "new genes" into a population by the transfer of individuals from one brood stock to another brood stock.

Mitosis The process of nuclear division in which the chromosomes are duplicated and distributed equally to the daughter cells so that each daughter cell has exactly the same chromosomal content.

Mixed strain A fish population, derived from a mixture of strains, that has not evolved sufficiently to be called a strain. (see **Strain**)

Monohybrid A cross between parents that differ with respect to a single specified pair of alleles.

Mosaic An individual that is composed of cells of two or more different genotypes.

Multiple alleles A series of three or more forms of a gene that can occur at a given locus.

Mutation A sudden heritable change in a gene or in the chromosome structure of an organism. Mutation is one mechanism for introducing genetic variability into a population.

Native brood stock A self-perpetuating brood stock maintaining itself in a natural environment (lake, pond, or stream) without supplementation by fish reared in hatcheries.

Natural brood stock A brood stock maintained in a natural environment by a combination of natural spawning and periodic stocking with fish reared in a hatchery.

Natural selection The selection pressure applied on a population by the environment. The population adapts to survive in that environment.

Oogenesis The physiological processes involved in the formation and maturation of female gametes (eggs).

Outbreeding Mating of individuals that are less closely related than randomly chosen individuals in the general population.

Overdominance The phenomenon of heterozygotes having a more extreme phenotype than either homozygote (monohybrid heterosis) for a specific trait. Overdominance generally refers to the situation in which AA' individuals are more "fit" than AA or $A'A'$ individuals.

Pleiotropy Multiple effects of a single gene in which the phenotypes of two or more apparently unrelated characters (traits) are affected.

Polygenes Several genes that individually may have relatively small effects but that collectively determine a quantitative trait.

Polymorphism The existence of two or more genetically different phenotypes of the same trait in an interbreeding population (e.g., color mutants or isozyme variants).

Polyploid A cell, tissue, or organism with more than two basic sets of chromosomes (e.g., triploid [$3n$] or tetraploid [$4n$]).

Population *1.* In genetics, a community of individuals that make up a single gene pool. *2.* In statistics, a hypothetical and infinitely large series of potential observations.

Post-kelt A spent or spawned-out salmon that has returned to the marine environment. This stage ends when the fish regains the weight it lost during spawning.

Post-smolt Stage during the first year at sea from time of departure from the river to the end of the first winter at sea.

Precocious parr Parr that are sexually mature (usually males).

Prepotency The ability of a parent to transmit characteristics to its offspring that make them more alike than usual.

Pre-smolt (silvery parr) Parr that have begun to transform to smolts—that is, undergone physiological changes before migration to the sea.

Progeny Descendants or offspring of individuals.

Progeny test A test of the genetic value of an individual based on the performance of its offspring.

Qualitative character A character determined by a small number of genes and in which variation is discontinuous, resulting in discrete phenotypic categories.

Quantitative character A character determined by many genes and in which variation is continuous, so that classification into discrete categories is not possible.

Quantitative inheritance Inheritance of a quantitative character that depends on the cumulative action of many genes, each of which produces a small effect.

Random Arrived at by chance without discrimination.

Random mating Condition where the probability of any one mating is equal to the probability of any other mating within the population (random union of gametes).

Recessive Member of an allelic pair that is expressed in the homozygous state but not expressed in the heterozygous state.

Reciprocal crosses Mating combinations in which the sources (families, lines, or strains) of male and female gametes are reversed – for example, the cross of a line A male mated to a line B female is reciprocal to the cross of a line B male mated to a line A female.

Recombination Formation of new combinations of genes as a result of segregation in crosses between genetically different parents. Also, the rearrangement of linked genes as a result of crossing over.

Recurrent parent The parent to which successive backcrosses are made in a backcross breeding program.

Repeat spawner Fish producing viable gametes for a second, third, or later spawning season after first sexual maturity.

Restoration The establishment of spawning populations adequate to optimally use the available habitat in watersheds where the species occurred historically but either does not now occur or occurs only in small numbers.

Salmon An Atlantic salmon regardless of age or state of sexual maturity that is older than the post-smolt stage.

Segregation The separation of homologous chromosomes at meiosis and the consequent separation of alleles and their phenotypic differences as observed in the progeny.

Selection Any natural or artificial process that favors the survival and reproduction of certain individuals.

Selection differential The difference between the average phenotypic value of a quantitative character in the total population and in the individuals selected to be parents of the next generation.

Selection intensity Proportion of the total population retained as parents for the next generation – usually expressed as a decimal fraction or percent.

Selection index A method for weighting different characteristics and choosing individuals to be retained as brood stock, based on a total score from all traits collectively.

Selfing Union of female and male gametes produced by the same individual.

Sex chromosomes Chromosomes that are different in the two sexes and together determine the genetic sex of the individual (usually called X- and Y-chromosomes).

Sex linkage Condition in which a gene affecting a specific phenotypic trait is on the sex chromosomes, resulting in sexually dependent inheritance of the trait.

Siblings (sibs) Brothers and sisters. Half-sibs are progeny with one parent in common (half-brothers and half-sisters); full-sibs are progeny with both parents in common (brothers and sisters).

Sibmating A brother-sister mating.

Single cross A cross between individuals with different genotypes – usually refers to a mating of two inbred lines.

Sire The male parent in animal breeding.

Smolt An actively migrating young salmon that has undergone the physiological changes to survive the transition from fresh water to salt water.

Smoltification The physiological process where young salmon adapt for the transition from a freshwater to a saltwater environment.

Somatic cells All cells of the body except gametes and the cells that produce gametes.

Spermatogenesis The physiological process involved in the production and maturation of male gametes (sperm).

Stock Fish spawning in a particular lake or stream (or portion of it) at a particular season, which to a substantial degree do not interbreed with any group spawning in a different place or in the same place at a different season.

Strain A fish population that exhibits reproducible physiological, morphological, or cultural performance characteristics that are significantly different from those of other fish populations of the same species, or a brood stock derived from such a population.

Strain cross The progeny resulting from the mating of individuals from two strains, one represented by the male parent and the other by the female parent.

Transition brood stock Fish population introduced into a hatchery from a natural environment as eggs or fish within the past two generations. The original brood stocks are identified as either F_0-native (derived from native brood stock) or F_0-natural (derived from natural brood stocks) during the initial generation in the hatchery. First generation progeny from F_0-native and F_0-natural brood stocks are identified as F_1-native and F_1-natural brood stocks, and later generations are simply identified as domestic brood stocks. (see **Captive brood stock**)

Tetraploid A cell, tissue, or organism with four basic sets of chromosomes ($4n$).

Translocation A chromosomal aberration that involves a change in the position of a segment of chromosome to another part of the same chromosome or to a different chromosome.

Triploid A cell, tissue, or organism with three basic sets of chromosomes ($3n$).

Truncation selection Process for choosing individuals to be retained as brood stock based on their expressed performance for a selected trait. Only individuals above the cutoff value are retained. The distribution of phenotypic values for the selected trait is therefore cut off, or truncated. (see **Mass selection**)

Viability Capability for living and developing normally.

Wild-type gene The allele commonly found in nature or the allele arbitrarily designated as "normal."

X-chromosome The sex-determining chromosome that is double in the homogametic sex and single in the heterogametic sex.

Y-chromosome The sex chromosome found only in the heterogametic sex.

Zygote The cell produced by the union of male and female gametes in reproduction. Also used to designate the individual developing from such a cell.

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16. Abstract (Limit: 200 words)

Anadromous runs of Atlantic salmon have been restored to the Connecticut, Merrimack, Pawcatuck, Penobscot and St. Croix rivers in New England by the stocking of more than 8 million smolts since 1948. Fish-breeding methods have been developed that minimize inbreeding and domestication and enhance natural selection. Methods are available to advance the maturation of brood stock, control the sex of production lots, and store gametes. Current hatchery practices emphasize the use of sea-run brood stock trapped upon return to the rivers and a limited number of captive brood stock and rejuvenated kelts. Fish are allowed to mature naturally, after which they are spawned and incubated artificially. Generally, 1-year smolts are produced, and excess fish are stocked as fry in headwater streams. Smolts are stocked during periods of rising water in spring. Self-release pools are planned that enable smolts to choose the emigration time. Culturists keep good records that permit evaluation of the performance of strains and the effects of breeding practices. As Atlantic salmon populations expand, culturists must use sound breeding methods that enhance biotic potential while maintaining genetic diversity and protecting unique gene pools.

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